ALLELOPATHIC IMPACT OF AGERATUM CONYZOIDES LINN. TOWARDS SOME CROP AND WEED PLANTS

ABSTRACT

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

BOTANY

BY

SMITA LAUR

DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
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Abstract

Abstract

Ageratum conyzoides a native of Central and South America has now become a dominant species in various tropical and subtropical countries including India. It is a destructive weed of agro ecosystems and invades cultivated fields and reduces growth and productivity of plants. Not only can the cultivated fields, the weed even be seen growing luxuriantly in other ecosystems also such as wastelands, grasslands and open unattended areas.

The weed grows very fast coupled with enormous seed production and provides selective evolutionary advantage to it. Due to long ecological amplitude, the weed shows great morphological variations and adaptability (Sauerborn and Kock, 1988). It is considered to be a common troublesome weed of agricultural as well as abandoned lands. It covers the public lands and thrives well on a variety of habitats in pastures, roadsides, rangelands, other open, ruined and waste sites or frequently disturbed areas and crop lands. It grows luxuriantly in rich, moist, mineral soils with high air humidity and tolerates shade. It forms monocultures and dense strands in the area soon after invasion because of its high regenerative potential and stolon formation.

Ageratum conyzoides is a major problem for farmers, ecologists, environmentalists, human beings and for animal scientists, especially in north India (Kumar and Singh, 1988). It over competes all other plant species upon, invasion resulting in adverse effects on natural vegetation and standing crops. Its invasion in crop fields hinders preparation of fields while ploughing. Due to its competitive nature, it results in reduction in availability of nutrients to crops thereby reducing productivity both quantitatively as well as qualitatively. Any field left fallow is likely to be invaded by this

weed and thus affecting the growth of other plants due to resource competition. Besides this, in the invaded areas, sustainability and soil health are deteriorated because of fast spreading stolons, utilization of nutrients and allelochemicals released by this weed. It, thus, damages the whole ecosystem. In uncultivated lands, upon invasion, it spreads vigorously covering the large areas forming pure cultures thereby reducing grazing area. It checks the growth of other grasses and weeds and thus causes thinning of floral diversity. As a result lesser land is available for the produce causing huge economic losses to farmers, shepherds and orchardists. It not only affects farmers, environmentalists and resource economists, but also adversely affects humans and livestock. People in contact with this weed suffer from nausea, giddiness, irritation and asthma. Livestock do not feed on it as it causes ulceration and toxicity. In other words, because of its fast spread weed also causes fodder famine.

Extensive growth and fast spread of *A. conyzoides* suggests that some interference mechanism is involved in its establishment. However, the cause of such interference is not known, although a few preliminary reports regarding phytotoxicity of *A. conyzoides* are available.

The plant height and dry biomass of test plants namely *Triticum aestivum*, *Brassica oleracea* var. *botrytis*, *Anagalis arvensis*, *Cicer arietinum*, *Melilotus alba*, *Phaseolus mungo*, *Oryza sativa* and *Polygonum pleubium* grown in rhizosphere soil of *A. conyzoides* were less than those grown in soil collected from the area free of *A. conyzoides* (control soil). However, there was no effect on germination. Further, when these soils were analyzed, rhizosphere soil of *A. conyzoides* was rich in available macro- and micronutrients compared to control soil. The rhizosphere soil contained significantly

more amounts of water-soluble inhibitors-phenolics. The inhibitory effect of rhizosphere soil of *A. conyzoides* suggested that the soil contained allelopathic / inhibitory substances in amounts sufficient to suppress the growth of test plants.

In order to find out whether these allelopathic substances are water-soluble or not, specific experiments were conducted. The aqueous extracts prepared from dried and powdered parts of the weed *viz.* stem, inflorescence, leaves, roots and above-ground parts of *A. conyzoides* were found to be inhibitory towards growth (plant height and dry biomass) of *Phaseolus mungo.* However, the phytotoxicity of extracts differed with different parts. In general, a concentration based effect of extracts was observed in the present study. The aqueous extracts from green leaves were found to be the most phytotoxic followed by roots or above-ground parts. On the other hand, aqueous extracts of inflorescence or stem exhibited lesser phytotoxicity. The aqueous extracts of each part were found to be rich in water-soluble allelochemicals

Further, the allelopathic effect of weed varied with different growth stages *viz.* plantlet stage, bud stage, flowering stage and seed stage. The extracts of different parts collected at flowering stage exhibited the maximum phytotoxic effect towards the growth of *P. mungo* whereas the aqueous extracts of weed collected at plantlet stage caused least phytotoxicity. These results suggested that maximum release of phytotoxic substances occur at flowering stage of the weed. In our study, significantly higher amount of phenolics were found to be present in the aqueous extracts prepared from different parts of *A. conyzoides* collected at flowering stage compared to those collected at any other growth stage of the weed.

Under natural conditions, weed residues / parts get mixed into soil. Therefore, an

experiment was performed where the effect of different parts of *A. conyzoides* was assessed in soil. For this, either different concentration of weed residues were directly added into soil or their extracts were mixed into soil. The growth performance of various plants was studied in these amended soils. The early growth of all test plants (*Anagalis arvensis*, *Brassica oleracea* var. *botrytis*, *C. arietinum*, *P. mungo* and *O. sativa)* was severely inhibited when grown in soils amended with different parts of *A. conyzoides* compared to unamended control soil. More growth retardatory effect was observed in soils amended with higher concentrations of powders or extracts. Further, these amended soils contained higher amounts of available nutrients as compared to control soil. Likewise, the phenolics were also found to be present in higher amounts in amended soils compared to control soil. The growth inhibitory effect of different parts of *A. conyzoides* in soil can thus, be attributed to release of phenolics in the soil. Maximum growth inhibitory effect was observed when residues or extracts of leaves were added into soil.

Since phytotoxicity of *A. conyzoides* may be altered in different textured soils, another experiment was planned where leaf powder was mixed in clayey, sandy, loam, clayey loam and sandy loam soil. In these soils, growth performance of *O. sativa* was observed. The seedling length and seedling dry weight of *O. sativa* was severely reduced in sandy soils amended with even lower concentration of leaf powder of *A. conyzoides.* At highest concentration of amendment, 90% inhibition in growth of seedlings of *O. sativa* was observed in these soils, Compared to sandy soils, phytotoxicity of *O. sativa* was the minimum in loam soil. Likewise, sandy soils mixed with leaf powder were found to contain very high amount of phenolics compared to other amended soils, thereby, indicating their role in retarding growth of *O. sativa.* The amount of phenolics in soils

varied with different texture and exhibited a direct relationship with observed growth retardatory effect. For example, more amounts of phenolics were detected where inhibition of *O. sativa* was more and *vice-versa.* Thus, soil texture greatly influences the allelopathic nature of weed.

Under natural conditions, the residues of weed undergo decomposition with time. In order to explore this, weed residues (leaf or root) mixed in soil in different ratios or alone, were allowed to decompose for two months. The length and dry weight of eight days old seedlings of *Brassica oleracea* var. *botrytis* were reduced to its maximum extent when allowed to grow in extracts of residues alone of either leaves or roots. The phytotoxicity of residues increased significantly for the initial 20 days as reflected through their effect on growth *Brassica oleracea* var. *botrytis.* It declined, thereafter; indicating the completion of decomposition. On the other hand, growth of *Brassica oleracea* var. *botrytis* seedlings remained unaffected in soil alone. Like the phytotoxicity, here also, the amount of phenolics increased initially for 20 days and decreased thereafter. In soil, where no change in growth of *Brassica oleracea* var. *botrytis* was observed, the amount of phenolics was also least. It is, therefore, concluded that during decomposition, significant changes occur in amount and nature of allelochemicals and hence phytotoxic nature of weed changes with time.

Further, the allelochemicals of *A. conyzoides* were also found to inhibit nodulation of *C. arietinum* (an important leguminous crop in India). Number and fresh weight of nodules of *C. arietinum* were significantly reduced, when grown in soil mixed with different parts of *A. conyzoides.* In lower concentrations, where nodulation occurred, leghaemoglobin content was significantly less, compared to control. In rhizosphere soil

of *A. conyzoides*, nodulation was completely inhibited compared to control. It is, however, difficult to say whether failure of nodulation is due to lack of root hair formation or inhibition of bacteria responsible for nodulation.

Lastly, attempt was made to identify various allelochemicals found in *A. conyzoides*. Eight phenolic acids were found to be present in organic extracts of different parts of *A. conyzoides* when analyzed through High Performance Liquid Chromatography (HPLC). These included *p*-coumaric acid, gallic acid, ferulic acid, hydroxybenzoic acid, anisic acid and syringic acid. In green leaves, all these were found to be present while only two *p*-coumaric acid and *p*-hydroxybenzoic acid were present in brown leaves. However, two remained unidentified. Their presence in different parts of weed suggests that these may be responsible for observed growth inhibitory effects on test plants

All these results show that *A. conyzoides* exert inhibitory effect on plant through the release of allelochemicals in soil from its different parts.

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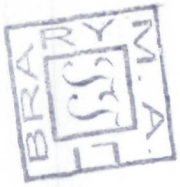

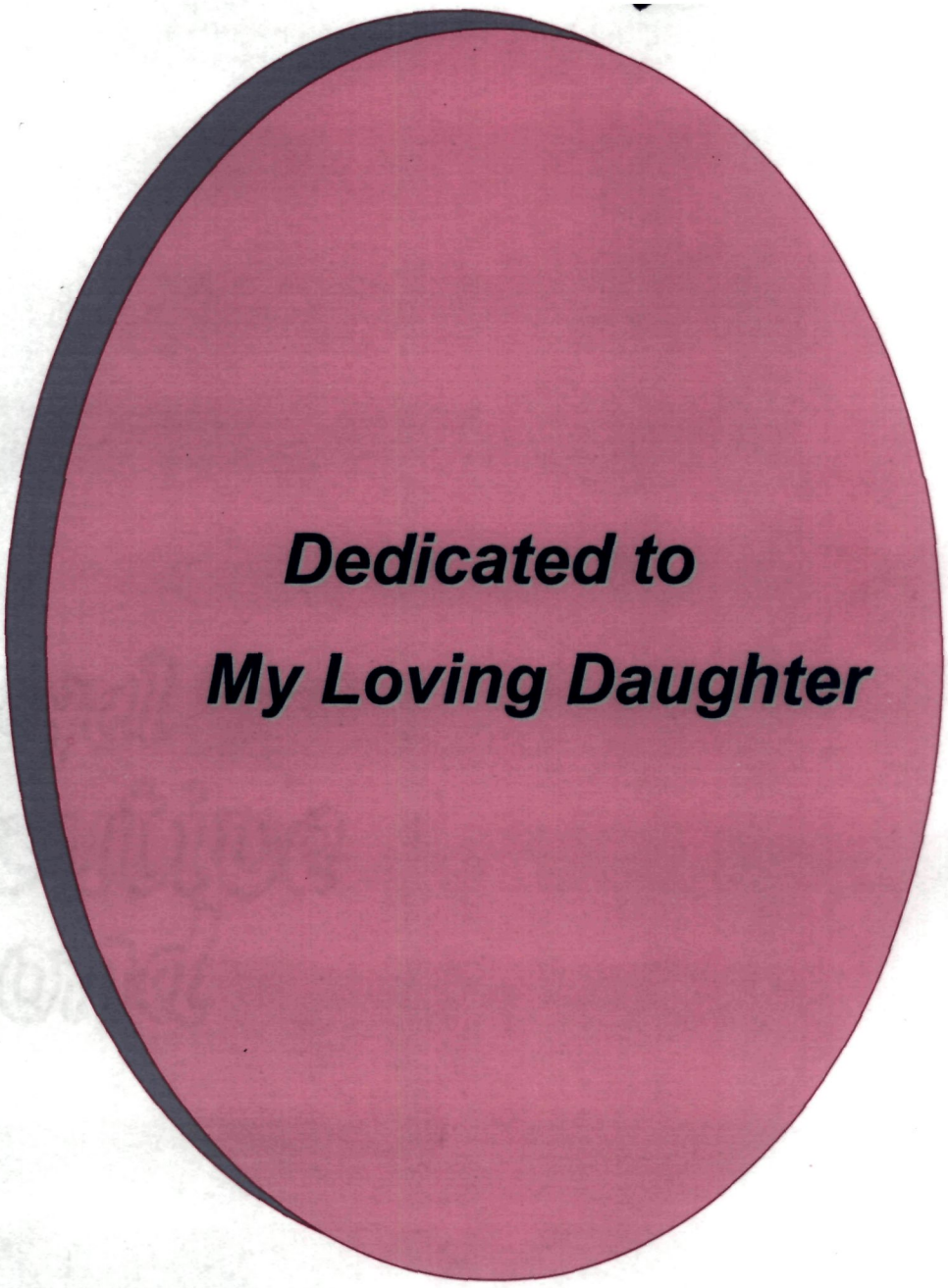
Dedicated to
My Loving Daughter

DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH-202002, INDIA

Dr. M.B. Siddiqui
M.Sc. Ph.D (Alig.)

Phone: 91-9412328937
Fax : 0571-2702016
Email : b.siddiqui@rediffmail.com
mbsiddiqui 6@hotmail.com

Certificate

This is to certify that the thesis entitled "Allelopathic impact of *Ageratum conyzoides* Linn. towards some crop and weed plants" submitted in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Botany, embodies a faithful record of the bonafide research work carried out by Ms. Smita Laur. No part of the thesis has been submitted for any other degree or diploma.

14.9.00

(Dr. M.B. Siddiqui)

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First, I bow in reverence to ***"Almighty God."*** *Omnipotent and Omnipresent, for it is indeed His blessing alone, which provided me enough zeal to complete this work.*

I deem it a great privilege in expressing my profound regard and deep sense of gratitude to my supervisor ***Dr. M.B. Siddiqui*** *whose professional acumen, meticulous suggestions, patient hearing, plausible and erudite thinking and obtrusive criticism helped me greatly in defying the conundrums of this work presented.*

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I have nothing to offer my family members but this small piece of work as a token of love and respect. My vocabulary fails to express my sincere feelings to my adorable ***Mummy*** *and* ***Papa*** *who sacrificed everything to give me the best education possible; to my sisters,* ***Neha*** *and* ***Kratika*** *for their loving and stimulating backup.*

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Smita Laur

(*Smita Laur*)

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ABBREVIATIONS / FORMULAE

µg	:	microgram	KH_2PO_4	:	Potassium hydrogen phosphate
µS	:	micro Siemens	$KMnO_4$	:	Potassium permagnate
$AgNO_3$	:	Silver nitrate	L	:	litre
Al	:	Aluminum	LHb	:	Leghaemoglobin
ANOVA	:	One-way analysis of variance	m	:	meter
			m.eq.	:	Milli equivalent
Ca	:	Calcium	Mg	:	Magnesium
$CaCl_2$	:	Calcium chloride	mg	:	milligram
Cl	:	Chloride	min	:	Minutes
Cm	:	Centimeter	ml	:	milliliter
Conc.	:	Concentrated	Mn	:	Manganese
Cu	:	Copper	mS	:	milliSiemens
$CuSO_4$	:	Copper sulphate	N	:	Nitrogen
DMRT	:	Dumcan's Multiple Range Test	Na	:	Sodium
			Na_2CO_3	:	Sodium carbonate
EC	:	Electrical conductivity	NaCl	:	Sodium chloride
EDTA	:	Ethylene diamine tetra acetic acid	$NaHCO_3$	:	Sodium bicarbonate
			NaOH	:	Sodium hydroxide
Fe	:	Iron	NH_3	:	Ammonia
$FeSO_4$	:	Ferrous sulphate	NH_4Cl	:	Ammonium chloride
g	:	Gram	NH_4OH	:	Ammonium hydroxide
h	:	Hour	nm	:	Nanometer
$HClO_4$	:	Perchloric acid	OC	:	Organic carbon
HCO_3	:	Bicarbonate ions	OM	:	Organic matter
HNO_3	:	Nitric acid	OP	:	Osmotic Potential
HPLC	:	High Performance Liquid Chromatography	ppm	:	Parts per million
			r	:	Correlation coefficient
K	:	Potassium	RT	:	Retention Time
$K_2Cr_2O_7$	:	Potassium dichromate	$SnCl_2$	:	Stannous chloride
K_2CrO_4	:	Potassium chromate	v/v	:	Volume/Volume
K_2SO_4	:	Potassium sulphate	var.	:	Variety
$K_3Fe(CN)_6$	:	Potassium ferricyanide	w/v	:	Weight/Volume
KCl	:	Potassium chloride	w/w	:	Weight/Weight
KCN	:	Potassium cyanide	Zn	:	Zinc
kg	:	Kilogram			

Introduction

INTRODUCTION

Ageratum conyzoides L. belongs to the family Asteraceae, commonly known as b:ll goat weed, is an annual, aromatic plant native to tropical America from where it has invaded to various parts of Southeast Asia including India (Wagner *et al.*, 1999), The generic name *Ageratum* is derived from Greek word *"ageras"* meaning *non-ageing,* referring to longevity of its flowers or whole plant whereas the specific name *"conyzoides"* is derived from Greek word *"konyz"* - the name of *Inula helenium* L. to which it resembles (Kissman and Groth, 1993). In India, it is spreading like a wild fire in many states especially Bihar, Bengal, Haryana, Himachal Pradesh, Punjab and Uttar Pradesh. It has become a serious weed especially in cultivated land of Uttar Pradesh where it is also known as "Ujaroo" in local language meaning destructive since due to its heavy infestation nothing is left in the invaded areas. As regards its entry into India, nothing is known with certainty. It is speculated to have entered with imported food grains.

Ageratum conyzoides is an annual aromatic herb, attains a height of 1m or even more. In plains, weed starts appearing in September-October and remains till April-May, whereas in hilly areas it grows throughout the year. The stem of this plant is erect, branched, cylindrical and decumbent. All the aerial parts of the plant are covered with small hairs known as trichomes. Leaves are opposite, ovate and triangular pubescent with the long petiole and trichomes are present on both the surfaces. The plant bears blue to violet inflorescence (capitulum) when young which turns white at maturity. Inflorescence is terminal and arranged in corymboraceme manner. Nearly 94772 seeds are produced per plant (Rodriguez and Cepero, 1984),

which are minute, black, light in weight, elliptical with pappus and have long dormancy periods. Besides being light in weight, the seeds can easily be disseminated to other areas resulting in quick spread of weed. Fruit is a typical achene.

The weed grows very fast coupled with enormous seed production and provides selective evolutionary advantage to it. Due to long ecological amplitude, the weed shows great morphological variations and adaptability (Sauerborn and Kock, 1988). It is considered to be a common troublesome weed of agricultural as well as abandoned lands. It covers the public lands and thrives well on a variety of habitats in pastures, roadsides, rangelands, other open, ruined and waste sites or frequently disturbed areas and crop lands. It grows luxuriantly in rich, moist, mineral soils with high air humidity and tolerates shade. It forms monocultures and dense strands in the area soon after invasion because of its high regenerative potential and stolon formation.

Ageratum conyzoides is a major problem for farmers, ecologists, environmentalists, human beings and for animal scientists, especially in north India (Kumar and Singh, 1988). It over competes all other plant species upon, invasion resulting in adverse effects on natural vegetation and standing crops. Its invasion in crop fields hinders preparation of fields while ploughing. Due to its competitive nature, it results in reduction in availability of nutrients to crops thereby reducing productivity both quantitatively as well as qualitatively. Any field left fallow is likely to be invaded by this weed and thus affecting the growth of other plants due to resource competition. Besides this, in the invaded areas, sustainability and soil health are deteriorated because of fast spreading stolons, utilization of nutrients and allelochemicals released by this weed. It, thus, damages the whole ecosystem. In

uncultivated lands, upon invasion, it spreads vigorously covering the large areas forming pure cultures thereby reducing grazing area. It checks the growth of other grasses and weeds and thus causes thinning of floral diversity. As a result lesser land is available for the produce causing huge economic losses to farmers, shepherds and orchardists. It not only affects farmers, environmentalists and resource economists, but also adversely affects humans and livestock. People in contact with this weed suffer from nausea, giddiness, irritation and asthma. Livestock do not feed on it as it causes ulceration and toxicity. In other words, because of its fast spread weed also causes fodder famine.

Extensive growth and fast spread of *A. conyzoides* suggests that some interference mechanism is involved in its establishment. However, the cause of such interference is not known, although a few preliminary reports regarding phytotoxicity of *A. conyzoides* are available. Allelopathy might play an important role in successful colonization of alien habitats by this weed. Aqueous root and shoot extracts of *A. conyzoides* inhibited seed germination and seedling vigour in soybean and maize (Singh *et al.*, 1989), and growth of rice and wheat (Jha and Dhakal, 1990; Prasad and Srivastava, 1991). A significant reduction in rice yield has been observed upon invasion by *A. conyzoides* (Roder *et al.*, 1998). Singh *et al.*, (2003b,c) observed that *A. conyzoides* adversely affects the growth and development of wheat, mustard and radish. Recently, it has been reported to drastically affect the diversity and density of the native herbal species in the Himachal Pradesh, India (Kohli *et al.*, 2004).

A number of chemical compounds belonging to various classes have been identified from *A. conyzoides* (Sharma and Sharma, 1995). These include flavonoids, alkaloids, chromenes, phenolics and essential oils (Gonzalez *et al.*, 1991; Sharma and

Sharma, (1995). The essential oils from flowers and leaves of the weed contain a mixture of 6-demethoxy ageratochromene and dimer of ageratochromene (Rastogi and Mehrotra, 1990). Among chromenes, precocene I and precocene II are biologically very active (Bowers *et al.,* 1976). These compounds act as anti-juvenile hormones and affect insect development (Borthakur and Baruah, 1987), and are also responsible for its allelopathic nature.

Several studies have indicated that volatile oil from this weed is also allelopathic in nature (Kong *et al.,* 1998a,b, 1999, 2002). Kong *et al.,* (1999) studied the inhibitory effect of fresh leaves and volatile oil of *A. conyzoides* on test plants and attributed this to the presence of precocenes and their derivatives and several monoterpenes and sesquiterpenes (Kong *et al.,* 1999, 2002). Precocene I, precocene II, 3,3-dimethyl-tert-butylindone and β-caryophyllene are the major constituents of volatile oils of *A. conyzoides* (Kong *et al.,* 1999). Allelochemicals of *A. conyzoides* act synergistically (Kong *et al.,* 1998b. 1999) and upon exposure to various environmental stresses, their allelopathic potential gets intensified (Josep and Joan, 1997; Kong *et al.,* 2000; Kong *et al., 2002).*

Kato-Noguchi (2001) reported that extracts of *A. conyzoides* inhibited germination and growth of *Amaranthus caudatus, Digilaria sanguinalis* and *Lactuca sativa.* Both radicle and hypocotyl lengths were severely reduced when test plants were grown in field soil previously infested with *A. conyzoides.* Not only fresh parts even the residues of the weed interfere with the growth of plants and other vegetation (Kalia, 1998; Kohli, 1998; Singh *et al.,* 2003,b). Hu and Kong (1997) reported that allelopathic potential of *A. conyzoides* varies with the test plant part, developmental stage and the habitat. Leaves were found to exhibit more inhibitory effect than stems

and roots (Xuan *et al.*, 2004).

Despite above preliminary reports, the mechanism of spread of weed at the cost of other plants still remains to be unknown. Further, there is lack of information as regards the changes in soil nutrient availability upon invasion of the weed. In order to fill this gap in knowledge, a study was planned to understand the interference of *Ageratum conyzoides* with test plants. The study was undertaken as per the following plan of work:

- Effect of *A. conyzoides* invaded rhizosphere soil on growth of different test plants.
- Comparative allelopathic effect of different parts of *A. conyzoides* on the growth and establishment of test plants.
- Dynamics of release of allelochemicals from different parts with respect to the age of the test plant
- Changes in the physicochemical properties of the soil in response to the allelochemicals of *A. conyzoides.*
- Nature of leachable inhibitors present in different parts (leaves, roots and above ground parts) of *A. conyzoides.*

Review Of Literature

REVIEW OF LITERATURE

The term 'weed' is generally used for any vascular plant that perpetuates itself in habitats where it is not required. Weeds have also been defined to be objectionable plants interfering with the activities and the welfare of man. Weeds are the excellent invaders of human-made habitats. Invasive weeds flourish at the cost of the native plants, degrade native environment and cause harm to any ecosystem; this is so particular with exotic weeds. Survey based on farmers perception indicate that weeds are the first or second most important agricultural constraints in various ecosystems including cultivated ones. Interference by weeds reduces the quality and quantity of productivity, causes health problems to human beings, changes landscape besides several other problems. Weeds are becoming a serious threat to the environment and economic well being globally and locally. The invasiveness of weeds may be attributed to their rapid growth rate; capability of forming dense, monocultures and thickets; high reproductive and regeneration potential; the release of allelochemicals; profuse seed production; effective seed dispersal; longevity of seed banks; long life span; adaptability to various ecological niches; stress tolerance; invasive potential; intense special competition; defense mechanism; herbicidal resistance; mimicry with crop seeds and seed polymorphism.

Weeds originate as pioneers of early stage of secondary succession that enable them to colonize, compete and regenerate rapidly in open and wasteland as well as in human-made habitats (Zimdahl, 1999), Weeds have co-evolved with crop plants since ages (Altieri and Liebman, 1988; Cousens and Mortimer, 1995; Zimdahl, 1999). These compete with them for food, light, space and water and serve as hosts for parasitic weeds; thus decline crop productivity. The presence of weeds on cultivated

land makes them less efficient for use by increasing costs of operations, production, harvesting and processing, diverting farmer's energy towards undesirable direction. In agricultural fields, weeds especially allelopathic ones reduce the yield of crops; deteriorate their quality, thus leading to financial losses (Kohli *et al.*, 1998; Qasem and Foy, 2001). Every year weeds cause nearly 12% loss of crop yield (Anaya, 1999). In US alone, weeds result in 12% yield loss, which costs nearly US$35 billion to control those (Pimentel *et al.*, 2001).

Weeds are competitively very strong as compared to other plants particularly those managed by man. Besides competition, allelopathy provides selective advantage to weeds. Interference - a term coined by Muller (1969) includes both competitive and allelopathic interactions. Of these, allelopathy is very common among weeds and has been well demonstrated in nearly 240 weeds (Qasem and Foy, 2001). However, under natural conditions, it is very difficult to differentiate the type of weed interference,

Weed Allelopathy

Allelopathy- a term first coined by Molisch in 1937 is a natural phenomenon which involves direct or indirect, beneficial or harmful (often harmful) effects of one plant on other through the release of chemicals that escape into the environment. Though the term first coined in 1937, yet it has a long history. In fact several reports, observations or documentations are available since Theophrastus (300BC), which indicates that the phenomenon of allelopathy was prevalent during very old time. Some important observations in the past were made by several workers (Theophrastus, 300BC; Culpeper, 1633; Young, 1804; de Candolle, 1832) Further, Singh *et al.*, (2001) divided the history of allelopathy into three major phases:

1) De Candolle phase- The period of late 18th and early 19th century

especially between 1785 and 1845.

2) Pre-Molisch phase-The period in the beginning of 20th centaury (from 1900-1920) known by the work of Pickering and Scheiner.
3) Post-Molisch phase- 1937 onwards which actually could progress since 1960 (Willis, 1997).

In communities different plant species may interact in a positive, neutral or negative manner. Positive inter-action includes obligatory or non obligatory mutualism. Rarely, the organism in a community remains neutral especially when canopies and roots of the plants occupy different niches. Negative interaction between the organism are, however, more common. The adverse impact of a neighboring plant in an association is termed interference (Muller, 1969). Putnam and Tang (1986) have been categorized interference as:

(i) Allelospoly

More commonly called competition which includes depletion of one or more resources acquired for the growth of organisms is an association.

(ii) Allelo-Mediation

Selective harboring of an herbivore that might feed on one species thus lending advantage to other (Szezepanshki, 1977).

(iii) Allelopathy

Alelopathy, the chemical of plant interference, is characterized by a reduction in plant performance in the association.

But the real recognition and understanding in allelopathy began in the last two decades. Research in this field has been intensified with as indicated by number of

publications on allelopathy. The actual and potential roles of allelopathy in agriculture have been extensively reviewed by many researchers and scientists. This is evidenced from several reviews and monographs (Rice, 1984; 1995; Qasem and Foy, 2001). Lots of work has been done on the allelopathic interactions of weeds with crops and other plants. For the sake of convenience, this work is being presented under two sections: (a) before 1998 and (b) after 1998 (in tabulated form).

(a) Some Important Reports on Allelopathic Weeds before 1998

Weeds exert their negative effects on plants by a number of processes viz. leachation from foliar and subterranean parts, volatilization from plant foliage, exudation from roots and subterranean parts as breakdown products through microbial decomposition (Rice, 1984). In general, decaying plant residues exhibit the most severe inhibition specially the early stages of decomposition. Studies of An *et al.*, (1997) have shown that with the increase in decomposition period, the phytotoxicity of an aqueous extract of *Vulpia* residue increased and was the maximum after 60 days of decomposition. A large number of compounds have been identified from these decomposing residues (An *et al.*, 2000). During decomposition process, a sustained toxicity may occur with the production and transformations of new toxic products (Patrick *et al.*, 1964). The water-soluble inhibitors are quickly released during decomposition. Phytotoxic effects of decomposing weeds residues on plants have been studied by many workers (Alsaadawi, *et al.*, 1990; Pratley and Irigrey, 1990; Rawat, 2002; Azania *et al.*, 2003). Schreiber and Williams (1967) studied that decomposing root residues of *Setaria faberi* Herrm. (giant foxtail), S. glauca (L.) Pal. (yellow foxtail) and *Digitaria sanguinalis* (L.) Scop, (crabgrass) severely reduced the corn growth. The degree of inhibition correlated with the amount of addition,

placement of residue and soil type. The inhibition increased with greater concentration of residue. At constant residue concentration, growth reductions increased in sandy soils (Einhellig, 1985).

Weeds exert negative impact on the symbiotic nitrogen fixation also by affecting both the host and the microsymbiont. Blum and Rice (1969) observed that nodules and leghaemoglobin content of red kidney bean *(Phaseolus vulgaris* L.) were severely reduced when grown in underneath soil of *Euphorbia supina* Raf. (prostrate spurge) and *Rhus copollina* L. Later on similar observation were also made with *Parthenium hysterophorus* L. by Kanchan and Jayachandra (1981). Growth of Rhizobium sp. and *R. leguminosorum* (Frank) Kirchner was inhibited by aqueous extracts of roots and shoots of *Andropogon virginicus* L. (broomsedge bluestem) (Rice, 1972).Weston and Putnam (1985) studied that root growth and nodulation of legumes *Glycine max* (L.)Merr.and *Phaseolus vulgaris* (L.) was severely inhibited by *Andropogon repens* (l.)Besuv.(quackgrass). Mallik and Tesfai (1988) studied soybean-rhizobia symbiosis. The extracts and residues of common weeds of pastures reduced nodulation in Soybean Halsall *et al.*, (1995) observed that aqueous extracts of weed residues, exerted allelopathic effects on subterranean clover and other species of pasture legumes resulting in poor root growth and nodulation.

Exudates of *Erica mediterranean* L. (mediterranean heath) inhibited germination and seedling growth of various crops as well as weeds (Alsaadawi *et al.*, 1990). Sundramoorthy and Sen (1990) studied the allelopathic effects of *Tephrosia purpurea* (L.) Pers. Volatile chemicals, water leachates of leaves, stems and roots and even soil collected near *T. purpurea* showed inhibitory effects towards *Pennisetum typhoides* (L.)Leeke,*Sesamum indicum* L. and *Cyamopsis tetragonolobus* (L.) Taub.

Chou *et al.,* (1991) observed the inhibitory effects of *Oryza perennis* Moench. towards root growth of *Brassica oleracea* var. *capitata.* Suseelamma and Raju (1992) observed that *Digera muricata* (L.) Mart. inhibited the germination and growth of *Macrotyloma uniflorum* (Lam.) Verde.Emergence and dry weight of *Capsicum frutescens* L., *Brassica chinensis* L., *Cucumis sativus* L., and *Brassica juncea* (L.) Czerniak were affected when debris of *Lantana camara* and *Chromolaena odorata* (L.) King and Robins (siam weed) were either placed on soil surface or incorporated into the soil (Sahid and Sugau, 1993). Wardle *et al.,* (1993) demonstrated that extracts, leachates and residues of *Carduus nutans* L. (musk thistle) inhibited the emergence and growth of pasture grass and legumes.Volatiles of *Calamintha ashei* (Weath.) Schinners (Ashei Savory) inhibited the germination of *Leptochloa dubia* (H.B.K.) Nees. Kato-Noguchi *et al.* (1994) studied that aqueous extracts of *Avena fatua* L. reduced seed germination; radicle and hypocotyl growth of *Lactuca sativa* L. Macharia and Peffely (1995) observed that root exudates of *Allium fistulosum* L. reduced the biomass of *Amaranthus spinosus* L. and *Kochia scoparia* (L.) Schrad. Qasem (1994b; 1995a,b,c) studied the allelopathy of a number of weeds viz. *Amaranthus blitoides* S. Wats., *A. gracilis* L., *A. retroflexus* L,, *Ammi majus* L, *Amigallis foemina* Miller, *Plantago lanceolata* L. and *Rumex crispus* L. against growth of *Triticum aestivum* L., *Pennisetum typhoides* (L.) Leeke,*Zea mays* L. and *Raphanus sativus* L. Many workers reported the allelopathic effect of *Lolium* spp. L. against *Trifolium* spp. L. and *Medicago* spp. L. (Takahashi *et al.,* 1991, 1993; Quigley *et al.,* 1990; Chung and Miller, 1995).Tang *et al.,* (1995) reported that growth of *Hordeum vulgare* L., *Raphanus sativus* L., *Oryza sativa* L. and *Glycine max* (L.) Merr. were reduced by *Cyperus rotundus* L.

(b) Reports on Allelopathic Weeds after 1998

Since 1990, there has been a spurt in reports on the allelopathic studies of weeds.It is primarily due to the availability of modern instrumentation for the identification and characterization of the allelochemicals involved. Besides, there has been increase in the number of studies demonstrating the phenomena under natural and managed conditions. After 1998, available reports on the allelopathic impact of the weeds in the agroecosystems are tabulated below in Table 1.

Table 1. List of weeds exhibiting allelopathic effects on other plants (reports after 1998).

Source	Target Plant	Part used and its effect	Reference
Acroptilon repens (L.) DC.	*Agropyron smithii* Rydb., *Bouteloua gracilis* (Willd. Ex Kunth) Lag., *Sporobolus cryptandrus* A. Gray	Soil with roots and litter reduced emergence, initial survival and root weight	Grant *et al.*, 2003
Ageratum conyzoides L.	*Triticum aestivam* L.	Extracts, leachates and volatiles of plant reduced germination	Chuihua *et al.*, 1999
A. conyzoides	*Aeschynomene indica* L. *Echinochloa crusgalli* (L.) *P. Beauv.*, *Monochoria vaginalis (Burm. f.) C. Presl. ex* Kunth, *Raphanus sativus* L.	Leaf residues inhibited growth and germination of *R. sativus* and *E. crus-galli* while it caused a complete inhibition of emergence of *M. vaginalis* and *A. indica*	Xuan *et al.*, 2004
Amaranthus spinosus L.	*Oryza sativa* L.	Aqueous extracts of leaves, roots and whole plant inhibited seed germination and seedling growth of O. sativa	Karim *et al.*, 2003
Argemone mexicana L.	*Lycopersicon esculentum* Mill.	Shoot extracts and decomposing tisuues caused inhibition in growth and fresh weight of seedlings	Shaukat *et al.*, 2002

Artemisia herba-alba Asso	*Helianthus squamatum* (A. Gray) A. Gray	Aqueous extract and soil below plant delayed germination	Escudero *et al.*, 2000
A. tridentate Nutt.	*Niconana attenuate* Torr.	Weed inhibited germination of test plant	Preston *et al.*, 2002
Asclepias syriaca L.	*Amaranthus retroflexus* L., *Chenopodium album* L., *Nicotiana tabacum* L., *Triticum aestivaum* L.	Root leachates and extracts severely reduced the growth of test plants	Kazinczi *et al.*, 1999
Atriplex bunburyana F. Muell., A. *codonocarpa* Paul G. Wilson	*Enchylaena tomentosa* R. Br., *Lactuca sativa*L., *Maireana georgei* (Diels) Paul G. Wilson	Aqueous and methanol extracts of leaves inhibited seed germination, root and shoot growth	Jefferson and Pennacchio, 2003
*Acacia retinodes Schltdl. Euphorbia serpens*L. and *Nicotiana glauca*	*Carrichtera annua, Conyza albida,* Lettuce cv. and Tomato cv.	Aqueous extract of whole plant inhibited the root length and shoot length	Dana and Domingo, 2006
A. conyzoides, Cynodin dactylon, Parthenium hysterophorus and *Solanum nigrum*	*Glycine max* (L.) Merril	Extract of whole plant inhibited the protein content, protein profile, seed germination and seedling length	Verma and Rao, 2006
Alliaria petiolata (Bieb) Cavara & Grande	*Geum laciniatum* Murr., G. *urbanum* L.	Root exudates retarded germination and growth of test plants	Prati and Bossdorf, 2004
Alnus nepalensis, Artocarpus heterophyllys and *Emblica officinalis*	*Oryza sativa, Phalaris vulgaris* and *Pisum sativum*	Aqueous extracts of leaf reduced the radicle growth of test plants	Kumar *et al.*, 2006
Amaranthus retroflexus, Chenopodium album, Erigeron Canadensis and Solanum nigrum	*Glycine max, Pisum sativum and Vicia sativa*	Extract of whole plant inhibited the seed germination	Marinov-Serafinov and Dimitrova. 2007
A. spinosus L.	*Oryza sativa* L.	Aqueous extracts of leaves, roots and whole plant	Karim *et al.*, 2003

		inhibited seed germination and seedling growth of O. sativa	
Ambrosia artemisifolia L.	*Amaranthus chlorostachys* L., *A. hypochondriacus* L., *A. retroflexus* L., *Chenopodium album* L.	Aqueous extracts of inflorescence caused reduced in growth	Bruckner *et al.*, 1993, 2003
A. annua	*Helianthus annuus, Lactuca sativa, Zea mays, Amaranthus ertroflexus, Echinochloa crus-galli and Lolium perenne*	Leaf extract reduced the germination of both (weed an crop) test plants	Koloren, 2006
A. harba-alba	*Anabasis setifera*	Aqueous extract of mature and immature fruit inhibited the germination percentage and seedling growth	Modallal and Charchafchi, 2006
A. siebery, A. nauchary and A. scoparia	*Amaranthus retroflexus*	Aqueous extract of leaf reduced the seed germination root length and shoot length of test plant	Samedani and Baghestani, 2005
A. annua	*Raphanus sativus, Brassica parachinensis, Brassica pekinensis and Oryza sativa*	Alcohol extracts inhibited the seed germination, seedling growth and root growth of test plant	Zhimei *et al.*, 2007
Brassica nigra (L.) Koch.	*Hordeum spontaneum* Koch.	Aquesous extracts and soil mixed with residues of each part reduced seed germination, hypocotyls length and seedling weight	Tawaha and Turk, 2003
B. nigra	*Medicago sativa* L.	Aqueous extracts of each part inhibited seed germination and seedling growth	Turk *et al.*, 2003
Cardaria draba (L.) Desv.	*Cucumis sativus* L., *Daucus carota* L., *Hordeum vulgare* L., *Oryza sativa* L., *Piper nigrum* L., *Triticum aestivum* L.	Extracts, leachates, exudates, volatiles and shoot residues inhibited germination and reduced growth of test plants	Qasem, 1999b, 2001

C. draba	*Agropyron cristatum* (L.) Gaertn., *C. draba, Pseudoroegneria spicata* (Pursh) A. Love, *Triticum aestivum* L.	Root extracts reduced germination and root growth of test plants	Kiemnec and McInnis, 2002
Centaurea diffusa Lam.	Grasses	Root exudates caused reduction in growth and density	Callaway and Aschehoug, 2000
C. maculosa Lam.	*Pascopyrum smithii* (Rydb.) A. Love, *Pseudoroegneria spicata* (Purch.) A. Love,	Soil, its extracts and residues of weeds caused growth inhibition in test plants	Olson and Wallander, 2002
Chenopodium album L.	*Phalaris minor* Retz.	Green manure inhibited test weed germination	Om *et al.*, 2002
C. murale L.	*Cucumis sativus* L., *Melilotus indicus* (L.) All., *Trifolium alexandrinum* L., *Triticum aestivum* L.	Soil beneath weed and root and shoot amended soil and shoots inhibited carbohydrates, dry matter, nutrient uptake, and soluble protein content	El-Khatib *et al.*, 2003
Chromolaena odorata (L.) King & Robins.	*Brassica rapa* L., *Capsicum fruitescens* L., *Cucumis sativus* L., *Piper nigrum* L., *Spinacea oleracea* L.	Weed debris and infested soil inhibited germination of test plants	Ambika, 1999
Convolvulus arvensis L.	*Phalaris minor* Retz.	Green manure of weeds inhibited germination	Om *et al.*, 2002
Cynodon dactylon (L.) Pers.	*Echinochloa crusgalli* (L.) P. Beauv., *Gossypium hirsutum L., Setaria verticllata* (L.) P. Beauv., *Zea mays* L.	Aqueous extracts reduced germination, total fresh weight and root length	Vasilakoglou *et al.*, 2005
Cyperus esulentus L.	*Avena sativa* L., *Citrus sinensis* (L.) *Osbeck, Lactuca sativa* L., *Sorghum bicolor* (L.) Moench., *Zea mays* L.	Weed residues caused reduction in germination, growth, fresh and dry weight	Bezuidenhout and Reinhardt, 1999

C. rotundus L.	*Oryza sativa* L.	Infested soil, extracts, leachates of leaves and tubers caused severe reduction of plant height, leaf area and weight	Quayyum *et al.*, 2000
Capsicum sp.	Chinese cabbage and Lettuce	Aqueous extract of whole plant reduced the seed germination rate, germination index and seedling shoot length of Chinese cabbage but promoted in Lettuce	Minghua *et al.*, 2007
C. murale L.	*Cicer arietinum* L., and *Pisum sativum* L.	Plant residue reduced the growth, nodulation and macromolecule content of test plants	Batish *et al.*, 2007
Croton bonplandianus	*Parthenium hysterophorus*	Leaf residue in soil inhibited the seed germination and seedling growth	Thaper and Singh, 2006
Croton bonplandianum	*Triticum aestivum* L.,*Brassica oleracea var. botrytis* L., *Vicia hirsuta, Melilotus alba* Medik.,*Vicia sativa*	Inhibited seedling,root length and dry weight	Sisodia and Siddiqui, 2008
Dittrichia viscose (L.) Greuter	*Malcolmia maritime* (L.) R. Br.	Soil mixed with leaf epicuticular exudates reduced root length and suppressed root hair	Levizou *et al.*, 2002
Echinochloa crus-galli(L.) P. Beauv.	*Oryza sativa* L.	Residues of weed reduced the yield of rice	Dilday *et al.*, 2001
Eupatorium adenophorum Spreng.	*Pisum sativum* L., *Triticum aestivum* L.	Weed residues inhibited growth of test crops	Song *et al.*, 1999
Euphorbia esula L.	*Pascopyrum smithii* (Rydb.) A. Love, *Pseudoroegneria spicata* (Pursh.) A. Love.	Soil, its extracts and residues of weeds caused inhibition of growth in test plants	Olson and Wallander, 2002
Evolvulus alsinoides L.	*Amaranthus caudatus* L., *Lepidiun sativum* L., *Phleum pretense* L.	Aqueous and organic extracts of shoots reduced germination and growth of plants	Kato-Noguchi, 2000
Festuca filiformis Pourret	*Digitaria sanguinalis* (L.)	Root exudates and plant extracts caused inhibition of	Bertin *et al.*, 2003a

	Scop., *Pleuraphis jamesii* Torr.	root growth	
Helianthus annuus L.	*Phalaris minor* Retz.	Green manure reduced the growth of weed	Om *et al.*, 2002
H. annuus	*Cyamopsis tetragonoloba* (L.) Taub., *Sorghum vulgare* Pers., *Zea mays* L.	Residues of preceding sunflower reduced crop density, weight of seed and total yield	Batish *et al.*, 2002c
H. annuus L., *Sorghum bicolor L. and Oryza sativa L.*	*Parthenium hysterophorus*	Aqueous extract of root and shoot reduced the germination and growth of test plant	Javaid.2006
H. annuus L., *Zea mays and Glycine max*	*Helianthus annuus*	Crops residue inhibited the plant height, root dry weight,top growth dry weight and total weight of test plant	Srisa, 2007
Imperata cylindrical (L.) Beauv.	*Brachiaria ramose* (L.) Stapf., *Cynodon dactylon* (L.) Pers., *Echinishloa crus-galli* (L.) P. Veauv., *Lolium multiflorum* Lam., *Sida spinosa* L.	Aqueous extracts of above ground parts and roots inhibited germination and seedling growth of test plants	Koger and Bryson, 2004
Iochroma australe Griseb.	*Chenopodium album* L., *Sorghum halepense* (L.) Pers.	Plant extracts caused reduction in germination and redicle length	Vaccarini and Bonetto, 2000
Juncus effuses L.	*Eleocharis obtuse* (Willd.) Schult., *Scirpus cyperinus* (L.) Kunth	Leachates of above ground parts exhibited reduction of chlorophyll a	Ervin and Wetzel, 2000
Lantana camara L.	*Eichhornia crassipes* (Mart.) Solms	Leaf Leachates and residues reduced germination and killed the weed	Saxena, 2000
Lolium rigidum Gaud.	*Dactylis glomerata* L., *Lolium multiflorum* Lam., *Medicago sativa* L.	Shoot extract inhibited radicle elongation	Emeterio *et al.*, 2004
Lepidium draba L.	*Glycine max* (L.)*Merr.*, *Hordeum vulgare* L., *Nicotiana*	Shoot extract inhibited radicle elongation.	Emeterio *et al.*, 2004

	tabacum L., *Triticum aestivum* L., *Zea mays* L.		
Lomandra longifolia	*Lactuca sativa*	Root exudates in soil reduced the growth of test plant	Asao, 2007
Macaranga tanarius (L.) Mull. Arg.	*Bidens pilosa* L., *Lactuca sativa* L., *Leucaena leucocephala* (Lam.) de With	Aqueous extracts and leaf residue mixed soil inhibited growth and weed density	Tseng *et al.*, 2003
Melilotus alba Medik.	*Triticum aestivum* L.	Dry matter of plant was reduced	Oudhia, 1999
M. indica (L.) All.	*Phalaris minor* Retz.	Green manure inhibited germination of test weed	Om *et al.*, 2002
Monochoria hastata (L.) Solms	*Oryza sativa* L.	Aqueous extracts of leaves, roots and whole plant inhibited seed germination and seedling growth of O.sativa	Karim *et al.*, 2003
Medicago sativa L. and *Vicia cracca* L.	*Amaranthus retroflexus* L. *Lolium perenne* L. *Ipomea hederacea [Pharbitis hederacea L.]* and *Portulaca oleracea* L.	Leaf and root extract inhibited the germination and growth	Koloren. 2007
M. alba Medik.	*Triticum aestivum* L.	Dry matter of plant was reduced	Oudhia, 1999a
Morus alba and *Toona ciliate*	*Cicer arietinum, Triticum aestivum, Zea mays, Phaseolus vulgaris* and *Glycine max*	Aqueous leachates reduced the germination radicle and plumule length of test plants	Kausal *et al.*, 2006
Ophiopogon japonicus Ker-Gawl.	*Cyperus difformis* L., *Echinochloa crus-galli* (L.) P. Beauv., *Monochoria vaginalis* (Burm.f.) C. Presl. ex Kunth	Aqueous extracts of underground parts inhibited seed germination, seedling growth and dry weight of test plants	Lin *et al.*, 2004
O. japonicus	*Brassica campestris* L., *Lactuca sativa* L., *Medicago sativa* L.,	Leaf residues and extracts inhibited emergence and dry weight, root and shoot length of test plants	Iqbal *et al.*, 2004
Oryza sativa	*Echinochloa* –	Aqueous extract of whole	Haibin, 2007

	crus-galli	plant and root exudates inhibited the seedling growth	
Parthenium hysterophorus L.	*Cicer arietinum* L., *Raphanus sativus* L.,	Residues and its and extracts caused reduction of seedling length and dry weight	Batish *et al.*, 2002a; Singh *et al.*, 2003a
P. hysterophorus	*Brassicu campestris* L., *B. rapa* L.	Root extracts alonw or amended in soil reduced seedling length and dry weight of test crops	Batish *et al.*, 2003b
Parthenium hysterophorus	*Cassia occidentalis, C. sophera* and *C. tora*	Aqueous extract of inflorescence,stem and leaf inhibited the seed germination and seedling growth	Rahman 2006a,b
P. hysterophorous	*Brassica rapa* L.	Aqueous extract of green leaf and flower reduced the seed germination ,survival,cotyledon area,leaf number,branch number, plant height,root weight, fertilization value and pollen viability of test plant	Prasad and Priyadarshani 2006
P. hysterophorous	*Lettuce*	Aqueous extract of leaf and flower inhibited seed germination and seedling growth	Wakjira *et al.*, 2005
P. hysterophorus	*Eragrostis tef* (Zucc.) Trotter	Aqueous extracts of flowers, stem, root and leaf inhibited germination	Tefera, 2002
Piper methysticum L.	*Echinochloa crus-galli* (L.)P.Beauv., *Lactuca sativa* L.	Residues inhibited emergence and growth of test plants	Hong *et al.*, 2002
Pluchea lanceolata (DC) C.B. Clarke	*Brassica juncea* (L.) Czerniak	Shoot height, pod number, and seed weight were reduced in weed incorporates soil	Inderjit, 2002
Pueraria thunbergiana (Sieb & Zucc.) Benth	*Barbarea vulgaris* R. Br., *Lactuca sativa* L., *Lolium perenne* L., *Phleum pretense* L.	Freeze-dried leaves inhibited germination and growth of roots and shoots	Kato-Noguchi, 2003a
Populus deltoides	Radish,French bean and mustard	Aqueous extract of leaf leachate reduced the germination and seedling growth of test plants	Khan *et al.*, 2006
Prosopis cineraria	Oryza sativa	Leaf extract inhibited germination and growth	Punjani *et al.*, 2006

R. bulbosus L.	*Echinochloa crus-galli (L.) P.* Beauv., *Ipmoea lacunosa* L., *Sida spinosa* L.	Residues inhibited the growth of test plants	Gander and Oliver, 1998
Ranunculus arvensis L. *R. asiaticus* L.	*Triticum aestivum* L.	Extracts,leachates,residues and even volatiles reduced growth and seedling length of wheat	Qasem, 1999a
Raphanus raphanistrum L.	*Cyperus esculentus* L., *Gossypium* spp. L., *Ipomoea lacunosa* L., *Senna obtusifolia* (L.) own & Barneby, *Zea mays* L.	Aqueous extracts of oven dried shoots suppressed germination and redicle growth of test plants	Norsworthy, 2003
Rheum emodi, Saussurea lappa and *Potentilla fulgens*	*Amaranthus caudatus, Phaseolus mungo, Phaseolus vulgaris, Elusine coracana*	Aqueous extract of whole plant reduced the germination of test plants	Tahir, 2007
R. raphanistrum	*Capsicum annuum* L., *Cyperus esculentus* L., *Lycopersicon esculentum* Mill.	Leaf margins of *L. esculentum* and *C. annum* turned necrotic after transplanting into soil amended with weed whereas in *C. esculentus* tuber production and dry weight were reduced to more than 80%	Norsworthy and Meehan, 2005
Ruta graveolens L.	*Cucurbita pepo* L. *var. pepo, Lens esculentus* Medik., *Raphanus sativus* L.	Weed residues inhibited the seed germination	Oliva *et al.*, 2002
Salvia syriaca L.	*Cucumis sativus* L., *Daucus carota* L., *Hordeum vulgare* L., *Oryza sativa* L., *Pennisetum typhoides* (L.) Leeke, *Piper nigrum* L., *Triticum aestivum* L.	Shoots residues and leachates, root exudates and volatiles from aerial parts caused reduction in seed germination and seedling growth of test plants	Qasem, 1999b, 2001

Sicyos deppei G. Don	*Cucurbita ficifolia* Bouche, *Phaseous vulgaris* L.	Leaf leachates caused inhibition of radicle growth	Cruz-Ortega *et al.*, 1998
Solidago altissima L.	*Oryza sativa* L.	Methanol extracts of roots and rhizomes inhibited the growth of rice	Ito *et al.*, 1998
Sorghum halepense (L.) Pers.	*Echinochloa crus-galli* (L.) P. Beauv., *Gossypium hirsutum* L., *Setaria verticillata* (L.) P. Beauv., *Zea mays* L.	Aqueous extracts reduced germination, total fresh weight and root length	Vasilakoglou *et al.*, 2005
Sphenoclea zeylanica Gaertn.	*Oryza sativa* L.	Aqueous extracts of leaves, roots and whole plant inhibited seed germination and seedling growth of O.sativa	Karim *et al.*, 2003
Schima superba	*Phoebe bournei*	Aqueous extracts of leaf and root inhibited the germination rate, fresh weight and dry weight of test plant	XiaoQing *et al.*, 2006
Solanum lycocarpum	*Sesamum indicum* L.	Aqueous extract of leaf and ground fruit tested seed germination and early growth in soil of test plant.	Aires *et al.*, 2005
Tamarindus indica L.	*Echinochloa crus-galli* (L.) P. Beauv., *Lactuca sativa* L., *Raphanus sativus* L., *Trifolium repens* L.,	Rhizosphere and amended soil and leaf extracts reduced radicle and hypocotyls growth and fresh a ıd dry weight	Parvez *et al.*, 2003a,b
Tithonia diversifolia (Hemsl.)A. Gray.	*Amaranthus viridus* L., *Oryza sativa* L., *Raphanus sativus* L., *Sorghum bicolor* (L.) Moench.	Weed infested soil inhibited seed germination and seedling growth of test plants	Tongma *et al.*, 1998, 2001
Tagetes minuta L. and *Eupatorium rugosum* Houtt.	*Aster scaber*, *Bidens bipinnata* and *Lotus corniculatus* var. *japonicus*	Aquesous extract of whole plant inhibited the root and shoot length	Jihyon and KewChelo, 2006
T. pretense, T.	*Lolium*	Water extracts of stem, leaf	CaiXia *et al.*,

repens, T. hybridum, Melilotus officinalis, Lucerne, Onobrychis viciifolia and *Vicia villosa*	*multiflorum*	and root inhibited the germination and seedling growth of test plants	2005
T. repens L.	*Abutilon theophrasti medic.* and *Echinochloa crus-galli* L.	Aqueous extracts of aerial parts and roots reduced the seed germination, root activity, respiratory rate and enzyme activities of test plants.	Ying *et al.*, 2006
Vicia villosa	*Veronica persica, Poa annua* and *Echinochloa – crus-galli*	Extract of leaves, roots and whole plant inhibited seed germination and seedling growth	XiaoXia *et al.*, 2007
Zilla spinosa (Turra) Prantl.	*Cotula cinerea* Delile, *Trichodesma africanum* (L.) Lehm., *Zygophylium coccineum* L.	Shoot and root extracts reduced percent germination and seedling length of test plants	El-Khatib and Abd-Elaah, 1998

Allelochemicals Involved in Weed Allelopathy

The chemicals with allelopathic potential are called allelochemicals or allelochemics (Whittaker and Feeny, 1971). Within the plant, these are synthesized as secondary metabolites. Although they are present in almost all parts of the plant i.e. roots flowers, seeds, stems, leaves, bark and even buds (Rice, 1974, 1984; May and Ash, 1990; Mahall and Callaway, 1991; Putnam and Tang, 1986) but their amount is more in leaves, roots and seeds (Rice, 1974). Plants synthesize a number of organic compounds that exhibit great diversity in structure and chemical nature. They belong to wide range of classes viz, alkaloids, coumarins, cyanogenic glucosides, flavonoids, phenolic acids, polyacetylenes, quinines and terpenoids (Rice, 1984, 1995). These are released into environment (generally rhizosphere soil) as water-soluble leachates from

different parts by different mechanisms. After release, allelochemicals are involved in a number of different metabolic processes (Mizutani, 1999; Waller *et al.*, 1999).

In the soil, these allelochemicals may get accumulated, regulate microbial community or change the physico-chemical properties of the soil by leaching (Whitehead *et al.*, 1981, 1982; Blum *et al.*, 1987; Blum and Shafer, 1988; Hinsinger, 2001). Heisey (1990a,b) studied immobilization of nutrients by addition of leaves of allelopathic plants. These are taken up by receiver plant, encourage beneficial symbiosis and inhibit the growth of competing plants species and are responsible for various interactions of plants with other neighboring and successional plants pathogens, insects and other herbivores (Bhowmik and Doll, 1982; Putnam, 1988; Einhellig, 1996; Nardi *et al.*, 2000). Their production, transformation or degradation occurs simultaneously in the soil. Several studies have reported that the amounts of allelochemicals vary with plant organs, growth stages and cultivar (Hasegawa *et al.*, 1992; Yamamoto, 1995; Nakamura and Nemoto, 1996; Premasthira and Zungsonliporn, 1996; Galfet and Pellisier, 1997; Wu *et al.*, 1999; Tongma *et al.*, 2001).

The quantitative characteristics of allelochemicals within the plant or after release are influenced by external environmental conditions such as stress factors while their quantity is affected by external as well as genetic factors (Dicke, 1094; Loughrin *et al.*, 1995; Pare and Tumlinson, 1997; Agrawal, 1998). Their availability i.e. their bioactive concentration in soil or their phytotoxic activity and hence, allelopathy in soil depends upon several factors viz, soil conditions, climatic conditions or fixation by soil component (Kobayashi, 2004). Soil texture modifies the action of allelochemicals due to their retention, transformation and transport by soil

particles (del Moral and Muller, 1970). Several studies have indicated modification of allelopathic potential by soil texture (Muller and del Moral, 1966; del Moral and Muller, 1969; Oleszek and Jurzysta; 1987).

A large number of allelochemicals have been identified exhibiting different chemical nature and structure. Allelochemicals bring about several changes in the target plants such as growth reductions, visible changes in the form of chlorosis, necrosis, blackening of tissue etc. The mode of actions of these allelochemicals is unknown, as little attention has been paid to this direction. Nevertheless some data is available in this regard. The effects of allelochemicals could be direct or indirect (Rice, 1984). For example, indirect effects of allelochemicals include inhibition of growth of microbial symbionts thereby reducing nodulation and consequently nitrogen availability (Rice, 1969). However, direct effects include reduction in growth and development of target plants. Allelochemicals may be selective in their action or plant may be selective in their responses. These considerations are complicated further by the presence of more than one active compound from a single plant.

Different allelochemicals have different modes of action. Batish *et al.*, (2003a) reported that parthenin (a sesquiterpene lactone from *Parthenium hysterophorus* L.) causes electrolyte leakage in *Phaseolus aureus.* Hell and Koster (2004) studied that juglone - an allelochemical from *Juglans nigra*, disrupts root plasma membrane H^+-ATPase activity and thus affect metabolism of root cells. Bais *et al.*, (2002) obtained racemic mixture of catechin from soil collected from spotted knapweed (*Centaurea maculosa*) fields. Later on these enantiomers were seperated from root exudates and studied, scientists found that (+)-catechin was responsible for Allelopathy while (-)-catechin provided anti-microbial properties to plant.

These natural compounds in purified or crude form possess potential to be used as bioherbicides and as germplasm source that could be used to enhance the weed suppression. They are effective at lower concentrations, possess greater ease of application, longer shelf life, a wider range of storage conditions, less space required for their storage and even a safer method to control weeds than synthetic herbicides as they are biodegradable and do not pollute environment. Several workers have studied the biochemical activities of allelochemicals to use them alternative to synthetic herbicides (Duke, 1986; Macias *et al.*, 1997; Hoagland, 2001; Duke *et al.*, 2002), pesticides, nematicides (Halbrendt, 1996; Soler-Serratosa *et al.*, 1996), fungicides, insecticides (Koul, 1992) and bactericides in agroecosystems. Besides these advantages, they play multiple ecological roles (Romeo, 1998) and may serve as phytotoxins, attractants, seed germination stimulants and inhibitors. Presence of a large variety of allelochemicals in soil also provides a diverse source of food energy for soil microbes. The allelopathic potential of many plants gets intensified by exposure to various environmental stresses (Einhellig, 1996) and induces phytochemical variation (Josep and Joan, 1997; Kong *et al.*, 2000).

Many workers have identified different allelochemicals from different weeds. Kil *et al.*, (1991) identified volatile essential oils (α-pinene, cioneole, camphor, (-)-trans-caryophyllene, β-myrcene, α-terpinine, γ-terpinine, (-)- thujone, bornyl acetate) from *Artemisia priceps*. Perez and Ormeno-Nunez (1991) identified scopoletin, courmarin, *p*-hydroxy benzoic acid, vanillic acid as major constituents of *Avena fatua* L. Dung *et al.*, (1992) identify α-thujone as major allelochemicals from *Artemisia vulgaris* L. The Emodin and physcion have been identified from *Polygonum sachalinense* F. Schmidt. (Inoue *et al.*, 1992). A number of phenolics acids viz. ferulic

acid, vanillic acid, *p*-coumaric acid and *p*-hydroxybenzoic acids have been identified from *Sasa cernaua* Makino (Li *et al.*, 1992). Mallik *et al.*, (1994) identified chlorogenic acid from *Chenopodium album* L. Thus, there is a long list of allelochemicals. Some of the potential identified allelochemicals from weeds are listed in Table 2.

Table 2: List of different allelochemicals identified from different weeds.

Source	Chemical nature	Chemical Name	Reference
Ailanthus altissima (Mill.) Swingle	Quassinoid	Ailanthone	Heisey, 1996; Heisey and Heisey, 2003
Alliaria petiolata (Bieb) Cavara	Thiocyanates	Allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC)	Vaughn and Berhow, 1999
Aphelandra squarrosa Nees and *A. Fuscopunctata* (Hook.) Vareschi	Hydroxamic	DIOBOA (2,4-dihydroxy-1,4-benzxazin-3(4H)- one) DIMBO 2,4 –dhyroxy-7-methoxy-1,4benzoxazin-3(4H)-one), HBOA 2-hydroxy-1, 4-benzoxazin-3(2H) One), HMBO (2-hydroxy-7-methoxy-1, 4-benzoxazin -3(2H)-one)	Baumeler *et al*, 2000
Centaurea diffusa Lam.	Sesquiterpene lactone	Cnicin	Fortuna *et al*, 2002
C. Maculosa Lam.	Flavonoid	(±)-Cetechin	Bais *et al.*, 2001
Cistus ladanifer L.	Phenolic acids	Ferulic, Cinnamic, 4-hydroxybenzoic, hydroxybenzoic, hydroxycinnamic and *p*-anisic acid, methyl propionate, azulence	Chaves *et al.*, 2001
Cyperus rotundus L.	Dicarboxylic acids, phenolic acid, fatty acid	Succinic, *p*-coumaric, ferulic, palmitic acis, stearic acid, α-hydroxy-hydrocinnamic acid, loeic acid, 4-hydroxy-hydrocinnamic acid, loeic acid, 4-hydroxy-benzeneacetic acid	Quayyum *et al.*, 2000
Desmodium uncinatum (Jacq.)	Isoflavones	Uncimanone A,B and uncimanone B,	Tsanuo *et al.*, 2003

DC.		Uncimanone C	
Empetrum hermaphroditum Hagerup	Tannins, phenolic acids	Batatasin-III	Gallet *et al.*, 1999
Fagopyrum esculentum Moench.	Carbpxamode	Fagomine, 4-piperidone, 2-piperidine methanol	Iqbal *et al.*, 2002
F. esculentum	Phenolic acids and fatty acids	Ferulic, caffeic, chlorogenic, palmitic, stearicm, arachidic and behanic acid	Tsuzui and Dong, 2003
Helianthus annuus L.	Flavnoids	Heliannone A,B	Rao *et al.*, 2001
Iochroma australe Griseb.	Withanolides	α.4, 7β, 20 α.trihydroxy-1-oxowitha-2, 5, 24-trienolide (2)	Vaccarini and Bonetto, 2000
Lantana camara L.	Phenolic acids	*p*-hydroxybenzoic acid, vanillic acid, caffeic, protocatechuic, transcinnamic gentistic syringic, ferulic, *o*-coumaric, *p*-coumaric, and salicylic acid	Ambica *et al.*, 2003
Leonurus sibricus L.	Phenolic acid	Caffeic acid	Mandal, 2001
Macaranga tanarius (L.) Mull. Arg.	Flavonoids	Nymphaeol-A,B and C, quercetin, abscisic acid, blumenol A,B and C, tarariflavanone A, tanariflavanone B	Tseng *et al.*, 2003
Polygonella myriophylla (Small) Horton.	Phenolic acids and Flavonoids	Gallic acid, quercetin hydroquinone, and rhamnetin	Weidenhamer and Romeo, 2004
Prosopis juliflora (Sw.) DC.	Amino acids and flavonoids	Tryptophan, syringin, (-)-lariciresinol	Naano *et al.*, 2002
Pueraria thunbergiana (Sieb & Zucc.) Benth.	Carotenoids	Cis-, trans-xanthoxin and trans, trnas-xanthoxin	Kato-Naguchi, 2003b
Rheum nobile Hook.f & Thomson	Flavonoids	Quercetin-3-o-glucoside, quercetin 3-o-galactoside, quercetin 3-o-rutinoside, quercetin 3-o-arabinoside, quercetin	Iwashina *et al.*, 2004
Solidago altissima L.	Polyactylene	DME (dehydromatricaria ester)	Ito *et al.*, 1998
Sphenoclea zeylanica Gaertn.	Dithiolane oxides	Zeylanoxide A, epizeylanoxide A, zeylanoxide B,	Hirai *et al.*, 2000

		epizeylanoxide B	
Vulpia myuros (L.) C. Gmelin	Phenolic acids	Benzoic acid, ferulic acid, salicylic acid, vanillic acid, syringic acid, succinic acid, catechol, hydrocinnamic acid	An *et al.*, 2000, 2001

Phenolic Allelochemicals

Phenolic compounds are one of the most widely distributed classes of secondary metabolites found in plants and soils (Rice. 1984: Einhelling. 1995: Pal. 1994). Phenolic acids, coumarins, flavonoids, quinines, and tannis collectively represent the plant phenolies. Except flavonoids, these are synthesized through shikimic acid pathway and their synthesis and/or accumulation is enhanced by environmental stresses (Kuiters, 1990). Phenolic acids form the largest group of plant phenolies. The quantity and composition of phenolic acids in the plants are uniquely different (Martens, 2002a, b) Phenolic acids, most often identified in extracts from plant tissues, include *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid (benzoic acid derivatives), and caffeic acid, *p*-coumaric acid, sinapic acid and ferulic acid (cinnamic acid derivatives).

Allelopathy is responsible for such growth behaviour many of the alleloehemicals are water soluble substance related into the environment through various ways. Environment factors (Reigosa *et al.*, 1999). According to Macias (1995; Chuihua, 2007), the application of naturally friendly to agricultural practice is a promising method to control weeds (Lavabre, 1991). Allelopathy was used (Chung *et al.*, 2001) to control bonyard grass [*Echinocloa Cruss-gallui*(P.)Beauv] in rice *(Oryza sativa* L.). Ahn and Chung (2000) as well as Chou (1999) used allelopathy as an ecological control tool against several weed in rice. The objective of this study was

therefore to determine the effect of Siam weed extract on the germination and the seedling performance of some crop and weed species. Multiple physiological effects have commonly been observed from treatment with many allelochemicals phenolies. These effects include decrease in plant growth, adsorption of water and mineral nutrients, ion uptake, leaf water potential, shoot turgor pressure, and osmotic potential caused by ferulic acid (Patterson 1981; Einhelling *et al.,* 1985a; Gerald *et al.,* 1992).These allelochemicals could either be phenolics (Guenzi and McCalla, 1962) or terpenoids (Macias *et al.,* 1996) and their action are dependent upon several biotic and abiotic factors (Einhellig, 1996).

Allelochemical and their Interaction with Soil Nutrients

Phenolic compound are the major allelochemicals found in soil infested with Quack grass (Whitehead *et al.,* 1982; Bobnick and Hagin, 1985).Quack grass *[Elytrigia* (L.) Nevaski] is a widespread weeds its root and shoot residues were shown to be phytotoxic to several crops including soybean [*Glycine max* (L.)Merr.], Navybean (*Phaseolus vulgare* L.) and alfalfa (*Medicago sativa* L.) Weston and Putnam (1986).

Upon release into the soil environment, phenolic forms component of the soil organic matter (Whitehead *et al.,* 1981, 1982; Inderjit, 1996; Martens. 2002b; Kobayashi, 2004). Phenolics occur in soil in three different forms-free reversibly bound and irreversibly bound. Phytotoxicity of many phenolic compounds greatly depends upon whether they are free or bound forms. Free phenolic compounds significant play a phytotoxic role in soil environment (Huang *et al.,* 1999). The most common phenolic acids found in soils are ferulic acid, *p*-coumaric acid, vanillic acid, protocatechuic acid (Whitehead *et al.,* 1982; Chou and Lee 1991; Li *et al.,* 1992), *p*-

hydroxybenzoic acid (Kuiters and Dennemen. 1987; Whitehead *et al.*, 1981), caffeic acid (Lodhi, 1976, 1978) and salicylic acid (Shindo *et al.*, 1978; Jalal and Read 1983).

Sunflower is a well known for its allelopathic compounds. Several phenols and terpenes have been reported in various cultivate of sunflower (Macias *et al.*, 2000a) .The allelopathic potential of three sunflower cultivars against weed known as most problematic in wheat including *Chenopodium polymorpha* L., *Rumex dentatus* L- and *Phalaris minor* Retz. The major source of allelochemicals in the rhizosphere is believed to be plants. These allelochemicals are generated directly or indirectly from precursor compounds released into the root zone and subsequently transformed through a biotic or biochemical reaction through the action of microbes or higher organisms (Tang *et al.*, 1989). Allelopathic interaction in soil environment depend greatly on the turn over rate of allelochemicals in the soil rhizosphere and their interaction with clay organic matter and other factors which change the physiochemical and biotic characteristics of the soil (Blum. 1995; Blum and Safer, 1988). Recent research by Blum and his laboratory have shown that soil texture, soil pH, organic carbon, available nitrogen are also very important in influencing uptake of allelochemicals and their ability to persist in the presence of soil microorganisms (Blum, 1995: Blum, 1998). Soil moisture dynamics can also influence the phytotoxicity of allelochemicals. Thus use soil as the medium of plant growth is an important aspect of such studies, as this brings condition closed to the natural situation and any change (Sorption chemical or microbial decay) in the bioactive concentration of allelochemicais upon entering the soil can be effectively demonstrated (Wardle and Nilsson, 1997; Blum *et al.*, 1999). Such studies using soil as the growth stimulate natural environment condition and carry great ecological

significance.

In a grassland ecosystem, accumulation of litter may influence soil pH, reduce ammonia losses, decrease wet nitrogen deposition and change the chemical composition of rainfall reaching the soil (Knapp and Seastedt, 1986; Facelli and Pickett. 1991). Soil pH is an important factor for uptake of allelopathic compounds (Blum, 1996). Incorporation of rice residue decrease both available nitrogen and concentration of soil action such as Ca, Zn, Mn and Na (Chou and Chou, 1979). Pal *et al.,* (1994) reported that the polyvalent elements Cu^{+2}, Mn^{+2} and Fe^{+3} facilitate the transformation of phenolic compounds. Similarly *p*-coumaric and vanillic acids had their great effects on barley *(Hordeum vulgare.* L.) growth under nitrogen and phosphorus deficient conditions.Protocatechuic acid form complexes with Fe and Al resulting in increased solubility and mobility of these metal ions (Shindo and Kuwatsuka, 1977a, b).

Weed-Crop Allelopathy

This discover that plant generated chemicals are involved in interference with other plants found a new concept to manage weed-crop allelopathy. The concept implies the use of crop cultivars with a built in herbicidal system capable of producing and releasing sufficient amounts of phytotoxic allelochemicals via root exudation that interfere with competing weed efficiently. Suppression of weed by root exudation of allelochemicals has proven to be of ecological relevance under field condition, however, the level is usually not sufficient to provide adequate weed control or the plant material is not available commercially (Perez and Ormeno-Nunez, 1993; Olofsdottere *et al.,* 1999; Fujii,2001).

A crop which is allelopathic should include the following characteristics:

(i) Affect the growth, productivity and yield of other crop.

(ii) May affect same crop growing in monoculture or grown in succession.

(iii) Cause soil sickness and imbalance of nutrients and microbial population, and

(iv) Can be exploited to selectively suppress weeds through various manipulations (Einhelling. 1985a; Batish *et al.*, 2001).

A large field experiment was conducted to evaluate the allelopathic potential of 1700 rice accession and growth of *Heteranthera limosa* and *Ammannia coccinea* was inhibited by 557 of these (Dilday *et al.*, 1994, 1998). It is obscure, however, whether these inhibitions were caused of only allelopathic interference because plant - to-plant interference is a complex combination of competitive interference for resources and allelopathy cannot be separated under field condition (Furest and Putnam, 1983; Olofsdotter *et al.*, 1999).

Aqueous extract of rice plant inhibited the growth of several plant species (Kawaguchi *et al.*, 1979) and aqueous extracts of decomposing rice residue inhibited root growth of Lettuce seedling (Chou and Lin, 1976). Several phenolic compounds, such as *p*-hydroxybenzoic acid, vanillic acis, *p*-caumaric acid and ferulic acids, were found in aqueous extracts of rice residue and straw (Kuwastsuka and Sindo, 1973; Chou and Chou 1979). [It is clear however, whether these compound is related from living rice plant]. Several workers have pointed out that separating allelopathic effect from other mechanisms interference would contribute a great deal to understand.Their relative importances rather than simply its existence (Nilsson, 1994; Ridenaur and Callaway), 2001, Weidenhamer, 1996). Weidenhamer *et al.*, (1989) have shown that allelopathy and resource competition can be distinguished of phytotoxic effects. Clearly, different mechanism of interference among plants may operate

simultaneously or sequentially in nature (Inderjit and DeMoral, 1997). Williamsom and Richardson (1988) suggested that statistical measurement of treatments in comparison to the control is required. Seed germination is widely used parameter in allelopathic bioassays (Rice, 1984; Williams and Hogland. 1982; Stowe, 1979. Rasmussen and Einhelling, 1977).

However, some workers found oven dry weight of radicle (Leather and Einhelling, 1985) and root length and root fresh weight to be statistically more accurate (Cope, 1982: Pederson, 1986).An aqueous extract of quack grass [*Agropyron repens* (L.) Beauv] was reported to contain a phytotoxic effective against germination and radical growth of several species of weed and crop plant (Weston *et al.*, 1987).

The soil concentrations (usually < 1 Mm) of many phenolic compounds do not reduce germination significantly (Rasmussen and Einhellig, 1977, 1979b; Einhellig and Rasmussen, 1978) suggested that in combination phenolic compound may interact synergistically to prevent seed germination. Crop residues are known to affect adversely the germination and growth of other during decomposition. The residue of preceding crops affect the performance other crops through the released allelochemicals Kimber 1973a,b; Guenzi and McCalla, 1966: Lodhi *et al.*, 1987; Thorne *et al.*, 1990; Kohli, 1993; Kong, 2006) reported the poor performance of cotton. The sugar beet, however, a number of soluble phytotoxic allelochemicals released by the residue accumulate in the soil and the crops roots may have a chance encounter with these chemicals leading to serious repercussion on the quality and quantity of crop yields (Einhelling, 1985a).

A number of crops have also been known to exhibit allelopathic property on other crops growing in succession or simultaneously or may even exhibit autotoxicity

(Einhelling, 1885a; Putnam and Weston, 1986; Chou, 1999; Anaya, 1999; Chuihua *et al.*, 2007). Among these especially the cover crops may be exploited for the purpose of weed management (Weston, 1996; Foley. 1999). The principal cause of crop auto toxicity or old root in soil that release phytoxins which may directly affect the succeeding crops, cause microbial imbalance, change in organic matter of soil increase ion leakage, disturb nutrient uptake and immobilization (Katznelson, 1972; Kimber, 1973a,b; Yu and Matsui, 1997). Some of the highly worked out important crops exhibiting crop autotoxicity include rice (Chou, 1995) wheat (Kimber, 1973a) maize (Yakle and Cruse, 1883, 1984). sugarcane (Chou. 1995) and several vegetable crops like cucumber, carrot funnel, watermelon, egg plant, tomato and even pea arc known to exhibit autotoxicity (Yu, 1999a)

Phenolic compounds have been identified as most common allelopathic agents than any other allclochemical substance and also responsible for growth inhibition. Some allelochemicals (mainly phenolic acids) identified from weed residue are listed below Table.3.

Table 3: Allelochemicals identified from weed residue.

Common Name	Botanical name	Allelochemicals (s)	Reference(s)
Arrow bamboo	*Sasa cernua* Makino	Ferulic, vanillic, p-coumaric and p-hydroxy benzoic acids.	Li *et al.*, 1992
Croton	*Croton bonplandianum L.*	Gallic acid, *p*- coumaric, cinnamic acid,anisic acid, *p*-hydroxybenzoic and ferulic acid	Sisodia & Siddiqui 2008
Buckwheat	*Fagopyrum esculentum* Moench	Ferulic, caffeic, chlorogenic, palmitic, stearic, arachidic and behenic acids.	Tsuzuki and Dong. 2003
Canada thistle	*Cirsium arvense* (L.) Scop.	Caffeic, ferulic, chlorogenic, *p*-coumaric, *p*-hydroxybenzoic and	Hussain *et al.*, 1987

		vanillic acids.	
Cocklebur	*Xanthium strumarium L.*	Caffeic, *p*-coumaric, *p*-hydroxybenzoic, chlorogenic and ellagic acids.	Inam *et al.*, 1987
Cogon grass or alang-alang	*Imperata cylindrical* (L.) Rausch.	Vanillic, *p*-coumaric, syringic chlorogenic acids, scopolin, scopoletin and isochlorogenic acid	Abdul-Wahab and Al-Naib, 1972; Eussen and Niemann, 1981.
Congress grass	*Parthenium hysterophorus L.*	Anisic, *p*-hydroxybenzoic, cinnamic, salicylic, *p*-coumaric, ferulic, caffeic, vanillic, chlorogenic, gallic, fumaric acids and sesquiterpene lactones (parthenin and coronopilin).	Kanchan, 1975; Kanchan and Jayachandra, 1980b
Curly dock	*Rumex crispus L.*	Phenolic compounds	Einhelling and Rasmussen, 1973
Giant rats tail grass	*Sporobolus pyramidalis* Beauv.	Ferulic. *p*-coumaric acids	Rasmussen and Rice, 1971
Jahanson grass	*Sorghum halepense L.*	Phenolic compounds mainly chlorogenic, *p*-coumaric acids and *p*-hydroxybenzaldehyde.	Rice, 1965
Japanese brome	*Bromus japonicus* Thunb. Ex Murr.	Phenolic acids	Rice and Parenti, 1967
Lamb squarter	*Chenopodium album L.*	Chlorogenic acid	Mallik *et al.*, 1994
Lantana	*Lantana camara L.*	*p*-Hydroxybenzoic, vanillic, *p*-coumaric, protocatechic, gentisic, caffeic, syringic, ferulic, *o*-coumaric, trans-cinnamic and salicylic acids.	Achhireddy *et al.*, 1985; Ambika *et al.*, 2003
Milk purslane	*Euphorbia supina* Raf.	Galli and tannic acids	Rice, 1965, 1969
Pellavatankio	*Commelina alyssum* (Mill) Thell.	Vanillic, *p*-hydroxybenzoic, ferulic acids.	Grummer and Beyer, 1960
Prickly glass wort	*Salsola kali* L.	Ferulic acid.	Lodhi, 1979b
Quack grass	*Agropyron repens* (L.) Beauv.	Succinic, *p*-coumaric, *p*-hydroxyphenylpropionic, phenylacetic, cinamic and 3-4	Lynch and Penn, 1980

		dihydroxyphenylpropionic acid	
Siam weed	*Chromolaea odorata* (L.) King &Robins	Phenolic acids and alkaloids	Ambika, 1999
Silver grass	*Vulpia* spp.	Pyragallol, catechlo, 3, 4-dimethoxyphenol, coniferyl alcohol, vanillic, p-coumaric, hydroquinone, protocatechuic, benzoic, p-hydroxy-benzoic, hydrocinnamic salicylic, gentisic, syringic, succinic, α-hydroxy-benzenepropanoic, p-hydroxybenzene propanoic, hydrocaffeic, p-hydroxyphenyl acetic, hydroferulic and ferulic acids.	An *et al.*, 2000a, b
Small Everlasting	*Antennaria microphylla* Rydb.	Caffeic acid, arbutin hydroquinone.	Maners and Galitz, 1986
Spanish health	*Erica australis* L.	Protocatechuic, vanillic, *p*-coumaric, *p*-hydroxybenzoic acids	Carballeira and Cuervo, 1980
Wild oat	*Avena fatua* L.	Vanillic, ferulic, caffeic, chlorogenic, *p*-coumaric, *p*-hydroxybenzoic, ellagic acids and scopoletin	Schumacher *et al.*, 1983; Qureshie *et al.*, 1987
Wild red rice	*Oryza perrennis* Moench nom. dub.	Phenolic acids	Chou et al., 1991
Yellow Fieldcress	*Rorippa sylvestris* (L.) Besser	Salicylic, *p*-hydroxybenzoic, vanillic, syringic acids, hirsuitin pyrocatechole isothiocyanates.	Yamane *et al.*, 1992a
Yellow nutsedge	*Cyperus esculentus L.*	Syringic, *p*-hydroxybenzoic, vanillic, ferulic, p-coumaric acids	Sanchez-Tames *et al.*, 1973

Materials & Methods

MATERIAL AND METHODS

For the present study pertaining to phytotoxic interference of *Ageratum conyzoides* against some plants, the relevant materials and details of methodology are discussed as under:

Collection of the Plant Material for the Study

Plants of *A. conyzoides* were collected locally from agricultural fields and other areas around the university campus of Aligarh Muslim University, Aligarh. The collection was made at different stages of growth *viz.* plantlet stage (nearly 5 cm), vegetative, flowering and seed stage. Different parts i.e. green leaves, stem, above ground parts (whole plant except roots), roots and inflorescence (wherever possible) were separated from *Ageratum* plants at each growth stage. Each part (of each growth stage) was separately dried, powdered and stored in labeled polyethylene bags till used. Likewise, above-ground parts, leaves (both green and brown) and roots were separated from mature *A. conyzoides* plants (at flowering stage), shade-dried, powdered and filled in polyethylene bags until used. For experiments with roots fresh collections were made.

Collection of Soil

(a) Rhizosphere Soil

Rhizosphere soil i.e. soil in and around the root system (approximately at 5-15 cm depth and 10 cm radius) was collected from *A. conyzoides* invaded agricultural fields or other areas of the University campus of Aligarh Muslim University, Aligarh The soil was collected from five different sites and at each site from five areas. The selected sites had the weed density of 49.0±10.13 plants/m^2

with average plant height of 75.53±10.52 cm. The root system reached up to 18.2 ± 5.3 cm with a spread area of about 415.97±12.5 cm^2.

Collection of soil was made from the upper 0-15 cm soil profile since 80% of the root system of weed is present in this zone.

(b) Control Soil

The soil was also collected from nearby areas free of *A. conyzoides* (at least 50 m away) to serve as control. The collected soil samples were shade dried, sieved through 2 mm sieve and filled in duly labeled polyethylene bags till further use.

Procurement of Seeds

For growth studies, adequate quantity of healthy and uniform seeds of crop plants (*Cicer arietinum* L., *Brassica oleracea* var. *botrytis* L., *Triticum aestivum* L., *Phaseolus mungo* L., *Oryza sativa* L.) and weed plants (*Anagalis arvensis* L., *Melilotus alba* Medik. , *Polygonum pleubium* R.Br.) were procured from Indian Agricultural Research Institute, New Delhi and National Research Centre for Weed Science, Jabalpur (M.P.) respectively.

Preparation of *A. conyzoides* Extracts

For each part of each growth stage, *2%* aqueous extracts were prepared from dried powered plant material (4% in case of above-ground parts). For this purpose, requisite amount of respective material was dipped in 1 L of pure water for 16-18 h. The contents were shaken well and filtered through two folds of muslin cloth followed by Whatman no. 1 filter paper. From the filtrate, dilutions were made with pure water to get lower concentrations of extracts.

Preparation of Amended Soils

Amendments in soils were done in two ways. In the first case, dried and powdered plant material (above-ground parts, leaves or roots) were directly mixed in soil. In other case, aqueous extracts prepared from plant material were added to the soil.

(a) Powder Amended Soils

In this case, requisite amount of dried powder (or residues) of plant material was mixed in soil so as to get concentrations of 0.5, 1, 2 and 4% (w/w). The contents were mixed well and used for growth studies. These were termed as powder amended soils.

(b) Extract Amended Soils

In this case, 500 ml of the extract of each concentration was added to 1 kg of soil. These were mixed well and dried. These were termed as extract amended soils.

(c) Root Amended Soils

For this, fresh roots were chopped and mixed into the soil at the rate of 5, 10, 20 g/kg soil. To these soils, 300 ml of pure water was added and kept for 24, 48 and 72 h.

Growth Studies

Under Laboratory Conditions (In Petri dishes)

Healthy and uniform seeds of test plants were used for growth study. Twenty seeds were dipped in each extract concentration and water (to serve as control) for 16 h. These were then equidistantly placed in a 15 cm Petri dish lined

with a thin layer of adsorbent, sterilized cotton and overlined by Whatman no. 1 filter circle moistened with 8 ml of respective extract solution or water. For each treatment, five replicates were maintained in a completely randomized block design. After eight days, seedling lengths of germinated seeds were measured and their dry weights were determined by keeping in oven at 75 °C for 48 h. The experiments were repeated.

In Experimental Dome (In Pots)

Growth studies were conducted in pots maintained at experimental dome. For this purpose, nearly 700 g of rhizosphere soil of *A. conyzoides,* all types of amended soils as well as control soils (unamended) were filled in duly labeled plastic pots (12.5 cm diameter). Five replicates were maintained for each plant. Five healthy and viable seeds of each plant were placed at 1 cm below the soil surface in each pot. The pots were placed in experimental dome. All pots were arranged in a completely randomized block design and adequately watered daily. The plants in each pot were allowed to grow for one month (in case of rhizosphere soil) or eight days (in case of other amended soil). After one week or month, number of plants emerged, were counted. These were carefully uprooted and their total plant height/seedling length and dry weight were determined by oven drying at 75 °C for 48 h.

Determination of pH of Extracts

The pH of each extract prepared from different plant parts was determined by immersing electrode of EcoScan digital pH meter (Eutech Instruments, Singapore), It was presented as mean of five replicates.

Determination of Conductivity of Extracts

The conductivity of plant extracts was measured with the help of an EcoScan Con 5 digital conductivity meter (Eutech Instruments, Singapore) by immersing its electrode into each extract. For this also, five replicates were maintained and expressed the mean values in μS or mS.

Determination of Osmotic Potential

Osmotic potential of extracts of plant material was determined using the following formula:

$$\text{Osmotic Potential} = 0.36 \times \text{Conductivity (in mS)}$$

Determination of Total Phenolic Content in Extracts

Total phenolic content in aqueous extracts was determined spectrophotometrically using Folin-ciocalteu reagent as per the method of Swain and Hillis (1959). To 1 ml of extract was added 1 ml of Folin-ciocalteu (50% diluted) reagent and 1 ml of 20% Na_2CO_3. It was allowed to stand for 30 min till blue color developed. This blue color was read at 700 nm on spectrophotometer against known concentration of ferulic acid. Pure water to which same reagents were added served as blank. For each test tube, five replicates were maintained. The amount of phenolics was expressed as μg/ml.

Determination of Leghaemoglobin Content in Nodules

The amount of leghaemoglobin was estimated as per method given by Wilson and Reisenauer (1963).

Reagents

Drabkin Solution: Dissolved 52 mg of potassium cyanide (KCN), 198 mg of

potassium ferricyanide [$K_3Fe(CN)_6$] and 1 g of sodium bicarbonate ($NaHCO_3$) in water and made the volume to 1L.

Extraction of Nodule Tissue and Estimation

In a pre-chilled pestle and mortar crushed 0.5 g of fresh nodule tissue in 3 ml of Drabkin solution. Transferred the contents to a 10-ml centrifuge tube and centrifuged the mixture (15 min at 500 x g) to separate nodule tissue and transferred supernatant solution into a 10-ml volumetric flask, Extracted the nodules thrice in a similar manner and combined the supernatants.Made the final volume of supernatant to 10 ml with Drabkin solution, mixed, and again centrifuged at 20,000 x g for 30 min. Read the absorbance of the cleared supernatant on spectrophotometer at 540 nm using a blank of Drabkin solution. Amount of leghaemoglobin was determined from the standard curve prepared with cyanmethehaemoglobin with concentrations 6.0, 4.0, 2.0, 1.0 and 0.5 mg per 10 ml of Drabkin solution.

Soil Analysis

(a) Determination of Soil pH and Conductivity

Soil extracts were prepared by mixing dried soil and pure water in the ratio 1:2 (w/v). For this, 20 g soil was mixed in 40 ml pure water. At least five samples each were kept for both *Ageratum* and control soil. The slurry of each soil type was stirred thoroughly for 1 h on electric shaker and kept undisturbed for 15 minutes. Aqueous extracts of respective soils were taken in beakers. The pH and conductivity of soil extracts were read directly with pH and conductivity meter, respectively.

(b) Organic Carbon

For the estimation of organic carbon in amended and unamended soils rapid

titration method of Walkley and Black (1934) was followed.

Reagents

a) Potassium dichromate solution: ($K_2Cr_2O_7$), 1N: Dissolved 49.04 g of potassium dichromate (AR grade) in pure water and made the volume to 1L.

b) Ferrous sulfate solution, N/2: Dissolved 139 g $FeSO_4.7H_2O$ (AR grade) in pure water and added 15 ml of conc. H_2SO_4 and diluted to 1L.

c) Ortho-Phosphoric acid: 85%.

d) *Diphenylamine: Dissolved 0.5 g diphenylamine (AR grade) in a mixture of* 100 ml conc. H_2SO4 and 20 ml pure water.

e) Conc. H_2SO_4 (AR grade): Not less than 96%.

Procedure

One g of dry soil sample was transferred into a 500 ml Erlenmeyer flask. To it was added 10 ml of $K_2Cr_2O_7$ followed by 20 ml of conc. H_2SO_4. The contents of the flask were shaken by hand for 1 minute and then kept undisturbed for half an hour. After half an hour nearly 200-225 ml of pure water was added. Thereafter, 10 ml of phosphoric acid and 1 ml of diphenylamine indicator solution were added to the flask. The content in the flask turned blue, which was titrated against N/2 $FeSO_4$ solution until the color changed to green.

Calculations

One ml of 1N $K_2Cr_2O_7$ is equivalent to 3 mg of carbon. The amount of carbon oxidized was expressed as percentage of soil as per the following formula:

$$\% \text{ Organic Carbon in soil} = \frac{\text{Titre value (ml)}}{\text{weight of the soil taken (g)}} \times 0.003 \times 100$$

Where, titre value = Total volume (ml) of 1N $K_2Cr_2O_7$ added - half the volume (ml) of N/2 $FeSO_4$used.

(c) Organic Matter

It is a function of carbon and thus was calculated as per the following formula:

$$\% \text{ OM} = \% \text{ OC} \times 1.724$$

(d) Available Nitrogen

Estimation of available nitrogen in the soil was done using alkaline potassium permanganate as per the method recommended by AOAC (Association of Official Agricultural Chemists, 1960).

Reagents

a) Potassium permanganate solution ($KMnO_4$): 0.32 %.

b) Sodium hydroxide solution (NaOH): 2.5%.

c) Conc. H_2SO_4: 0.02N.

d) Sodium hydroxide solution (NaOH): 0.02N.

e) Methyl red indicator: Dissolved 0.5 g methyl red in 100 ml ethyl alcohol.

Procedure

To 20 g of dry soil taken in 800 ml Kjeldahl distillation flask, was added 20 ml of pure water, 100 ml of 0.32% $KMnO_4$ and 100 ml of 2.5% NaOH. To avoid bumping, few glass beads and 5-7 ml liquid paraffin were added to Kjeldahl flask and then fitted in distillation apparatus. The end of delivery tube was dipped in a 250 ml conical flask containing 20 ml of 0.02N H_2SO_4 and 2-3 drops of methyl red indicator. Tap water was passed through condenser and contents of flask were heated. The pure ammonia gas was collected into the conical flask until its volume became 100 ml.

Thereafter, conical flask containing the distillate was removed and titrated the excess of H_2S0_4 against 0.02N NaOH till the end point i.e. from pink to yellow color, was reached. Noted the volume of NaOH used.

Calculations

Weight of soil taken = 20 g

Volume of 0.02N H_2SO_4 taken = 20 ml

Volume of 0.02N NaOH taken = X ml

Volume of 0.02N acid used for absorbing NH_3 = (20-X) ml

Available nitrogen = (20-X) × 20 kg/ha

(e) Available Phosphorus

For the estimation of available phosphorus in the soil, the method described by Olson *et al.*, (1954) was followed.

Reagents

a) 0.5NSodium bicarbonate ($NaHCO_3$): Dissolved 42 g of $NaHCO_3$ (AR grade) in 1L of water.Adjusted the pH to 8.5 with the help of 10N NaOH.

b) Ammonium molybdate solution: Twenty five gram of ammonium molybdate (AR grade) was dissolved in 200 ml of water, Diluted 275 ml of conc. H_2SO4 with 500 ml of water and cooled. Poured the ammonium molybdate solution into acid by stirring and made the final volume to 1 L.

c) Activated charcoal: To remove any adhering phosphate, treated 0.5N $NaHCO_3$ with Darco G.60.

d) Stannous chloride: Dissolved 100 mg of standard stannous chloride (AR grade) in 5 ml of conc. HCl by warming in a test tube. Diluted it to 10 ml with

water. To it added ½ inch layer of liquid paraffin and wrapped a brown paper around test tube to prevent oxidation.

e) *p*-nitrophenol solution (0.5%): (yellow in alkaline medium and colorless in acidic) was used as an indicator.

f) Conc. H_2SO_4 (1+4): Mixed one volume of conc. H_2SO_4 and four volumes of pure water.

g) Standard: Dissolved 2.1965 g of KH_2PO_4 (AR grade) in pure water. To it, was added 25 ml of 1+4 H_2S0_4 and diluted to 1L resulting in a solution containing 500 ppm of phosphorus. From this further dilutions were made.

Procedure

To 5g of dry soil sample taken in a 250 ml stoppered glass flask, added 1-2 teaspoon full of Darco G.60 and 100 ml of 0.5N $NaHCO_3$. The solution was shaken for 30 min on electric shaker and filtered through Whatman filter paper no. 41. Twenty ml of filtrate was taken in a 50 ml volumetric flask. To it was added 2-3 drops of *p*-nitrophenol indicator. This yellow colored solution was neutralized with 1+4 H_2SO_4 and further diluted to 40 ml. To this was added 2 ml of ammonium molybdate solution and the final volume was made to 50 ml. Transferred the solution from volumetric flask to 100 ml conical flask. Developed the color by adding 0.12 ml of $SnCl_2$. Transmittance was read at 660 nm between 6-12 min. The available phosphorus, expressed in ppm, was determined from standard curve.

Calculations

Weight of soil sample = 5 g

Volume of 0.5N $NaHCO_3$ used = 100 ml

Volume of filtrate taken = 20 ml

Final volume made for color development = 50 ml

Total dilution = 20 × 2.5 = 50 times

Concentration of phosphorus read from standard curve = Y ppm

Available phosphorus in the soil (ppm) = Y × dilution factor = Y × 50 ppm

(f) Available Potassium and Sodium

For the estimation of K and Na, method as described by Bower and Gschwend (1952) was followed.

Reagents

a) Ammonium acetate solution: Dissolved 77.09 g of ammonium acetate in pure water and made the final volume to 1L.Adjusted the pH to 7.0 with the help of acetic acid or ammonium hydroxide solution.

b) Working standard: Dissolved 1.9068 *g* dry KCl (AR grade) and 2.5419 g of NaCl (AR grade) in pure water and made the volume to 1L, separately. The concentration of solution was 1000 ppm and it was diluted to produce a suitable range between 0 and 100 ppm of potassium or sodium.

Procedure

To 1 g of soil sample taken in 100 ml conical flask added 25 ml of ammonium acetate solution. Shook the contents for 5 min on an electric shaker and then filtered through Whatman no. I filter paper. The filtrate was put in an atomizer of the flame photometer and read the values.

Calculations

Weight of soil sample taken = 1g

Volume of ammonium acetate added = 25 ml

Reading of flame photometer for test solution = A ppm

Dilution factor = $25/1$ = 25 times

Available K or Na in the soil (ppm) = A × 25

(g) Available Calcium and Magnesium

Estimation of Ca and Mg was done using disodium dihydrogen ethylene diamine tetra acetic acid (EDTA) as per the method given by Black (1973).

Ca + Mg

Preparation of Extracts

Soil extracts were prepared in 1:5 (w/v) ratio. For this, 50 g soil was mixed with 250 ml of pure water in 500 ml in conical flask. The contents were shaken for 1 h on electric shaker and kept undisturbed for 24 h. The next day, soil extracts were filtered through Whatman no. 1 filter paper and kept in plastic reagent bottles for further use. These extracts were used for the analysis of available Ca, Mg, Cl and HCO_3 and determination of total phenolics.

Reagents

a) EDTA solution (0.01N): Dissolved 2 g of EDTA in 900 ml H_20 and made the final volume to 1L.

b) Ammonium Chloride-Ammonium Hydroxide buffer: Dissolved 67.5 g of NH_4Cl (AR grade) in 570 ml of NH_4OH. To it was added 400 ml of pure water and adjusted its pH to 10. Then made the final volume to 1L with water.

c) Eriochrome Black T Indicator: Dissolved 0.5 g of Eriochrome Black T and 4.5 g hydroxylamine hydrochloride (AR grade) in 100 ml of 95% ethyl alcohol.

Procedure

To 5 ml of soil extract, was added 20 ml of pure water and 1 ml of NH_4Cl-NH_4OH buffer followed by 3-4 drops of Eriochrome black T indicator. The solution in flask that turned wine-red coloured, was titrated with 0.01N EDTA solution till the end point i.e. from wine-red to blue was reached.

Calculations

$$\text{Milli equivalent Ca + Mg (per litre or 200g soil)} = \frac{\text{Volume of EDTA used (ml)}}{\text{Volume of soil extract taken (ml)}} = X$$

$$\text{m. eq. Ca + Mg / 100 g soil} = \frac{X}{2} = Y$$

$$\text{Percent Ca + Mg (g/100g soil)} = Y \times 10 = A$$

Ca only

Reagents

a) Sodium hydroxide (4N): Dissolved 160 g of NaOH (AR grade) in pure water and made the volume to 1L.

b) Ammonium purpurate (Murexide) indicator: Mixed 0.5 g of ammonium purpurate with 100 g of powdered potassium sulphate thoroughly.

Procedure

Took 5 ml of soil extract in a 100 ml conical flask and added 20 ml of pure water, 0.25 ml of 4N NaOH and about 50 mg of ammonium purpurate indicator. The solution in the flask turned orange and was titrated with 0.01N EDTA solution till the end point i.e. from orange to purple color was reached,

Calculations

$$\text{Milli equivalent Ca (per litre or 200 g soil)} = \frac{\text{Volume of EDTA used (ml)}}{\text{Volume of soil extract taken (ml)}} = B$$

$$\text{m. eq. Ca /100g soil} = B/2 = Z$$

$$\text{Percent Ca (g/100g soil)} = Z \times 10 = C$$

Mg only

Milli equivalent Mg /100g soil = Y – Z Percent Mg (g/100 g soil) = A – C

(h) Available Bicarbonates

Estimation of available HCO_3 was done by using standard sulphuric acid as per the method given by Black (1973).

Reagents

a) 0.1N conc. H_2SO_4.

b) Methyl orange indicator: Dissolved 0.1 g of methyl orange indicator in 100 ml of 95% ethyl alcohol.

Procedure

To 25 ml of soil extract added 2-3 drops of indicator. The solution in the flask turned yellow, which was titrated against 0.1N H_2SO_4 till the end point i.e. from yellow to rose red was reached.

Calculations

$$\text{Milli equivalent of } HCO_3 \text{ (per litre or 200g soil)} = \frac{\text{Volume of } 0.1\ H_2SO_4 \text{ used (ml)}}{\text{Volume of soil extract taken (ml)}} \times 100 = B$$

$$\text{m.eq. of } HCO_3 \text{ /100g soil} = B/2 = C$$

$$\text{Percent } HCO_3 \text{ (g/100g soil)} = C \times 10$$

(i) Available Chlorides

Estimation of Cl from the soil was done by using potassium chromate as per the method given by Black (1973).

Reagents

a) N/35.5 Silver nitrate ($AgNO_3$) solution: Dissolved 4.785 g of silver nitrate in 900 ml pure water and the volume was made to 1L. It was stored it in amber colored bottle to avoid oxidation.

b) Potassium Chromate (K_2CrO_7) indicator: Dissolved 1 g of K_2CrO_7 in 100 ml of H_2O.

Procedure

To 20 ml of soil extract added 1 ml of K_2CrO_7. Titrated this yellow colored solution against $AgNO_3$ till brick red precipitates appeared.

Calculations

$$\text{Milli equivalent Cl (per litre or 200 g soil)} = \frac{\text{Volume of } AgNO_3 \text{ used (ml)} \times \text{Normality of } AgNO_3}{\text{Volume of extract taken (ml)}} \times 1000$$

$$\text{m.eq. Cl / 200 g soil} = \frac{\text{Volume of } AgNO_3 \text{ used (ml)} \times 1 \times 1000}{35.5 \times 20} = B$$

$$\text{m.eq. Cl/100 g soil} = B/2 = C$$

Percent Cl (g/100 g soil) = C × 10

(j) Available Iron, Manganese, Zinc and Copper

DTPA (Diethylene triamine penta acetic acid) was used to extract available form of these micronutrients from the soil. The amount (in ppm) of

these micronutrients in the extracted solution was determined on Atomic Absorption Spectrophotometer (AAS).

Reagents

DTPA Extracting Solution: Dissolved 1.967 g of DTPA, 1.47 g $CaCl_2.2H_2O$ and 13.3 ml (14.92 g) of reagent grade TEA (triethanalamine) separately in pure water and then mixed the three solutions and made the volume to about 900 ml with pure water. Adjusted the pH of solution to 7.3 with the help of 1N HCl and made the final volume 1L.

Procedure

To 20 g of soil sample, added 40 ml of DTPA extracting solution and shook the mixture for 2 h on a rotary shaker. Filtered the solution through Whatman filter paper no. 42 and read the values on Atomic Absorption Spectrophotometer (AAS). For each micronutrient analyzed, a separate standard curve was prepared and the concentration of each micronutrient (ppm) was calculated from the respective standard curve.

Calculation

Weight of the soil taken = 20 g

Volume of DTPA solution added = 40 ml

Dilution factor = 2

Reading of micronutrient on AAS = X

Concentration read against X on standard curve = Y ppm

Content of micronutrient in soil = Y × 2 ppm

(k) Determination of Total Phenolic Content from Soil

For this, 1:5, w/v soil extracts (prepared above) were used. The amount of total phenolics was determined from 1 ml of these extracts using Swain and Hillis (1959) method as already detailed.

Statistical Analysis

In all the experiments, five replicates of each treatment were maintained and treatments were arranged in a completely randomized block design. For each experiment, statistical analysis was applied using software programmes like SPSS (ver. 11.0, Origin 5, and Microstat). For determining the significance of a single treatment with control (paired treatment), student's 2-sample *t*-test was applied. In case of experiments involving more than two treatments, analysis was done with one way analysis of variance (ANOVA) followed by separation of means using Duncan's Multiple Range Test (Duncan, 1955). In some experiments where treatments were governed by two factors simultaneously, two-way ANOVA was also applied to determine the interaction between factors and treatments. The significance of data was checked at 5 or 1% level, wherever applicable. In experiments involving effect of different concentration on a given parameter, values of correlation coefficients were also calculated.

Experiments

EXPERIMENT – 1

Objective

To study the effect of soil collected from *Ageratum conyzoides* invaded fields on growth of some test plants.

Hypothesis to be tested

The weed *Ageratum* releases water-soluble phytotoxins into the soil of its infestation through leachation or by decomposition. These phytotoxins accumulate in bioactive concentrations in the rhizoshpere (active root zone of soil) and affect the growth and development of plants.

Parameters studied

Seed germination, plant height and dry weight of 8 test plants species namely *Cicer arietinum* L., *Brassica oleracea* var. *botrytis* L., *Triticum aestivum* L., *Anagalis arvensis* L., *Melilotus alba* Medik., *Phaseolus mungo* L., *Oryza sativa* L., and *Polygonum pleubium* R.Br. sown in soil collected from *A. conyzoides* invaded area were studied. Some physical and chemical properties of *Ageratum* invaded field soil and uninvaded control soil were also compared.

Methodology

Rhizosphere soil (5 – 15 cm profile) was collected from *A. conyzoides* invaded agricultural fields selected near the campus of Aligarh Muslim University, Aligarh. Likewise, soil was collected from an area at least 50m away from *Ageratum* infested site to serve as control. Collected soil was shade dried, sieved through 2mm sieve and filled in polyethylene bags for further use.

Uniform and healthy seeds of the test plants. *Cicer arietinum* L., *Brassica oleracea* var. *botrytis* L., *Triticum aestivum* L., *Anagalis arvensis* L., *Melilotus alba* Medik., *Phaseolus mungo* L., *Oryza sativa* L., and *Polygonum pleubium* R. Br. were used for growth studies. Control soil or *Ageratum* invaded field soil was filled in plastic pots. In each pot so filled, 5 seeds of either of the test plants were sown. Five replicates were maintained for each test plant. One week after sowing, pots were thinned to 2 visually uniform plants. After 30 days, plants were uprooted and plant height and dry weight was determined. At the time of termination of the experiment, soil samples from five randomly selected pots (control as well as test pots) were analyzed for pH, conductivity, organic carbon, organic matter and total phenolics and available N, P, K, Ca, Mg, Na, Cl and HCO_3 Cu, Zn, Mn, and Fe following methods mentioned in section material and methods. Each experiment was repeated data of each test plant species were put to *t*-test analysis at $p < 0.05$ and 0.01.

Results

(a) Germination

All seeds of each of the test plants tested germinated in soil collected from *Ageratum* invaded fields as well as the control. Since, there was no change in germination, data have not been presented.

(b) Plant Height

In general, plant height of test plants emerging from the seeds sown in rhizosphere soil of *A. conyzoides* was shorter than those of control. The plant height of *T. aestivum* was found to be 56.97±1.71 cm in control. Compared to this its plant height in *Ageratum* invaded soil was only 40.97±1.76 cm, exhibiting a reduction of

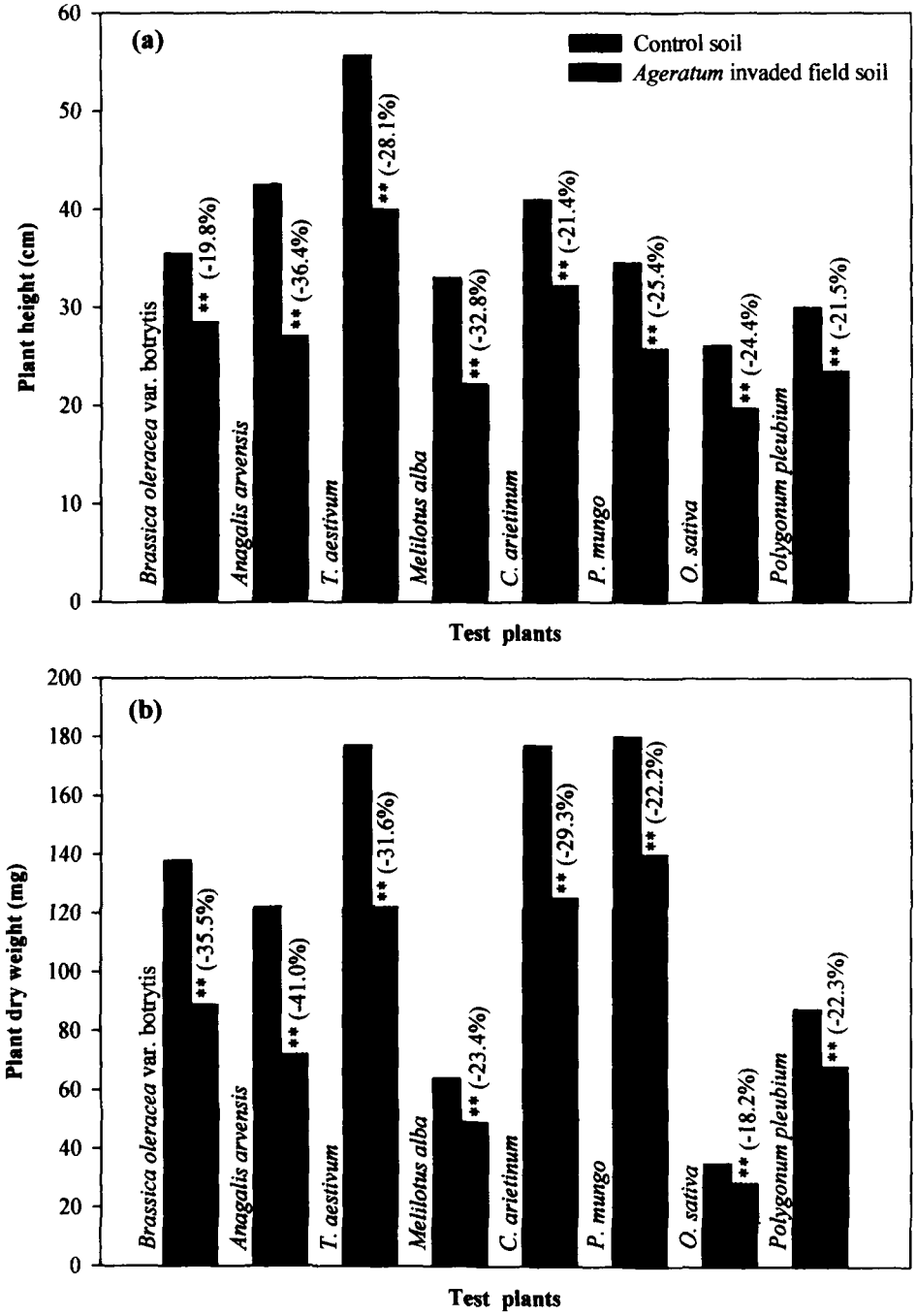
about 28.1% over control (Fig 1.1a). This reduction was significant statistically. In *C. arietinum* height of plant growing in soil collected from *Ageratum* invaded field was 31.84±1.22 cm compared to 40.49±0.99 cm in control showing a reduction of 21.4% (Fig 1.1a).

Brassica campestris var. *botrytis* and *Anagalis arvensis*, a significant reduction in plant height was noticed. It was about 36.4% over control in *Anagalis arvensis* and 19.8% in *Brassica oleracea* var. *botrytis* .In *Anagalis arvensis*, plant height in *Ageratum* invaded field soil was only 27.0±1.01 cm compared to 42.47±1.66 cm in control soil. In *Brassica oleracea* var. *botrytis* however, the difference between plant heights in control and *Ageratum* invaded field soil was not much, although it was statistically significant at $p<0.01$ (Fig 1.1a). Likewise, in *Melilotus alba* over 30% reductions in plant height was observed (Fig. 1.1a). Plant height was also reduced in *Ageratum* invaded field soil compared to control. In *P. mungo* plant height in *Ageratum* invaded field soil was 25.85±0.37 cm compared to 34.66±0.79 cm in control showing a reduction of 25.4%. Likewise in *O. sativa* and *Polygonum pleubium* more than 20% reduction in plant height was noticed (Fig 1.1a).

(c) Plant Dry Weigh

Like plant height, dry weight of all test plants was measured to be less in *Ageratum* invaded field soil compared to control soil. In *T. aestivum*, dry weight of plants in *Ageratum* invaded field soil was reduced by 31.6% i.e. it was 123.4±1.1 mg compared to 180.4±0.79 mg in control. In *C. arietinum* also, a significant reduction in plant dry weight was noticed. It was 180.25±4.6 mg in control whereas 127.48±2.76 mg in *Ageratum* invaded field soil (Fig. 1.1b).

Fig. 1.1. Plant height (a) and dry weight (b) of test plants one month after sowing in soil collected from *Ageratum* invaded area or control



* and ** represent significant difference in value from respective control at $P<0.05$ and 0.01, respectively

In *Anagalis arvensis* and *Brassica oleracea* var. *botrytis* nearly 40.4 and 30.7% reduction in plant dry weight was observed (Fig 1.1b). In fact in *Anagalis*

arvensis reduction in plant dry weight was the maximum in all the test plants. In *Melilotus alba* the dry weight in control was measured to be 64.5±2.25mg whereas it was 49.34±1.11 mg in *Ageratum* invaded field soil. So, a reduction of about 23.6% from that of control was noticed. In *P. mungo* dry weight was reduced by 23.03% i.e. it was measured to be 141.11±0.67 mg in *Ageratum* invaded field soil compared to 183.33±0.48 mg in control soil (Fig. 1.1b). Similarly, reduction in dry weight was also noticed in *Polygonum pleubium* and *O. sativa* and it was 18.2% in *O. sativa* and 25.9% in *Polygonum pleubium* (Fig. 1.1b). In *O. sativa* reduction in dry weight was significant only at $P<0.05$, whereas in other crops it was significant at $P<0.01$ as well as 0.05, Maximum reduction in plant height and dry weight was also observed in *Anagalis arvensis.* In *Melilotus alba* more than 30% reduction in plant height was noticed. As regards dry weight, the reduction was the maximum in *Anagalis arvensis* followed by *T. aestivum* and *Brassica oleracea* var. *botrytis.*

(d) Soil Properties

Soils collected from both control and *Ageratum* invaded field soil were also analyzed for various soil properties and status of the nutrients available. The pH was more in uninvaded soil (nearly 7.5) than in *Ageratum* invaded field soil where it was near neutral (~ 6.9). Conductivity on the other hand was measured to be three times more in *Ageratum* invaded filed soil compared to that of control soil (Table 1.1).

Likewise, osmotic potential was also three times more in *Ageratum* invaded field soil compared to control soil. Besides pH, conductivity and osmotic potential (OP), amount of total phenolics, organic carbon (OC) and organic matter (OM) were also measured to be more in the *Ageratum* invaded field soil compared to that in

control. All values in *Ageratum* invaded field soil were statistically significant at P<0.01 and 0.05 (Table 1.1).

Table 1.1 Changes in some soil properties due to invasion of *A. conyzoides*.

Parameter	Uninvaded control soil	*Ageratum* invaded field soil
pH	7.51±0.03	6.88±0.09**
Conductivity (μS)	130.33±5.45	390±8.0**
OP (bars)	46.92±1.96	140.4±2.88**
Phenolics (μg/g soil)	7.55±0.14	30.07±1.032**
OC (%)	0.53±0.07	1.14±0.0**
OM (%)	0.914±0.12	1.965±0.0**

** indicates significant from control at *P*<0.01

The amount of various soil nutrients *viz.* N, P, K, Ca, Mg, Na, Cl, HCO_3, Cu, Zn, Mn and Fe were also assessed in two types of soils. A significant difference with respect to all nutrients was observed between *Ageratum* invaded field soil and control soil. In general, the amount of various nutrients was found to be more in *Ageratum* invaded field soil than control (Table 1.2). The content of nitrogen was found to be nearly 2.4 times more in *Ageratum* invaded field soil compared to control. Amount of available P was 2.3 times more compared to control. The amount of K was significantly more in *Ageratum* invaded field soil compared to control soil (Table 1.2).

Likewise, the contents of Ca, Mg and Na were significantly more in *Ageratum* invaded field soil compared to control (Table 1.2). The amount of Mg was 9.0 g/100g soil compared to only 4.83 g/100g soil in control soil. The amount of Cl was also more in *Ageratum* invaded filed soil compared to control and. it was 4-times more compared to control Further, the amount of HCO_3, Cu, Zn, Mn and Fe was also more

Table 1.2 Changes in some macro- and micronutrients due to invasion of *A. conyzoides*.

Nutrients	Uninvaded control soil	*Ageratum* invaded field soil
N (kg/ha)	98.57±7.87	234.0±0.0*
P (ppm)	87.0±4.24	199.36±3.68**
Ca (g/100g soil)	7.83±0.29	14.67±0.72*
Mg (g/100g soil)	4.83±0.29	9.0±0.0**
Na (ppm)	82.5±0.0	118.33±2.89**
K (ppm)	291.67±12.58	561.67±12.58**
Cl (g/100g soil)	3.25±0.11	14.08±0.0**
HCO_3 (g/100g soil)	13.13±0.58	23.0±1.0**
Cu (ppm)	0.686±0.01	0.886±0.1*
Fe (ppm)	3.27±0.14	6.67±0.06**
Mn (ppm)	5.803±0.18	6.79±0.11**
Zn (ppm)	4.89±0.02	2.2±0.21**

** and * indicate significant from control at $P<0.01$ and $P<0.05$ respectively.

in *Ageratum* invaded field soil except Zn, which was measured to be less compared to control (Table 1.2).

Discussion

It is clear from the experiments that the growth of the test plants namely *C. arietinum*, *Brassica oleracea* var. *botrytis*, *T. aestivum*, *Anagalis arvensis*, *Melilotus alba*, and *P. mungo*, *O. sativa* and *Polygonum pleubium* was significantly affected when grown in the soil collected from *Ageratum* invaded fields (rhizosphere soil) compared to control. Both, plant height and biomass accumulation were significantly reduced in *Ageratum* invaded field soil. Maximum effect was observed in case of *Anagalis arvensis* where both plant height and dry weight was reduced to maximum

extent compared to other plants. On the basis of plant height of the test plants the decreasing order of sensitivity of plants appeared to be *Anagalis arvensis* > *Melilotus alba,* > *T. aestivum* > *P. mungo* > *O. sativa* > *Polygonum pleubium* > *C. arietinum* > *Brassica oleracea* var. *botrytis,* whereas on the basis of dry weight, the decreasing order of sensitivity was observed to be *Anagalis arvensis* > *T. aestivum* > *Brassica oleracea* var. *botrytis,* > *C. arietinum* > *Polygonum pleubium* > *Melilotus alba* > *P. mungo* > *O. sativa.* The studies indicate that some inhibitors are present in the rhizosphere soil of *Ageratum* that adversely affects the early growth of test plants compared to control. In order to find out the reasons, the soil from *Ageratum* dominated and *Ageratum* free areas was compared for various soil properties and nutrient status. In this context, soil properties *viz.* pH, conductivity, osmotic potential, phenolics, organic carbon, organic matter etc. were assessed and compared. As already mentioned in the results, though changes were observed in pH, conductivity and osmotic potential in the two types of soil, these changes, however, are unlikely to make any difference in the growth. Further, the amount of organic carbon and organic matter were improved from that of control. So observed growth inhibitory effect could not be attributed to these reasons. Likewise, amount of all the nutrients (whether macro- or micro- or ions) was more in *Ageratum* invaded field soil compared to control soil and hence they are not responsible for growth retardatory effects of test plants. On the other hand, the phenolics - a well known group of secondary metabolites (Harborne, 1989; Seigler, 1996; Mizutani; 1999), were found in appreciable amount in rhizosphere soil from *Ageratum* invaded area compared to control: Several studies have indicated that these phenolics are responsible for growth retardatory effect on test plants thus causing appreciable injury to the growing plants

(Rice, 1984, 1995; Qasem and Foy, 2001; Weston and Duke, 2003; Kong *et al.*,2006; Yang *et al* ., 2006).

Rhizosphere soil is an active root zone of soil, which is densely populated and where most of the biotic interactions among microorganisms occur (Walker *et al.*, 2003). It is also an abundant source of organic material on which fauna and flora are dependant for food (Ryan and Delhaize, 2001; Souza-Filho *et al.*, 2006). Most of the chemicals especially allelochemicals released from plants also accumulate in this zone. These may be released by roots as exudates or from above-ground parts through leachation or microbial degradation. Roots, however, are known to serve as one of the major source of organic chemicals released through root exudation. These exudates may contain a diversity of chemicals that regulate the biotic communities of soil besides its physical and chemical properties. These also inhibit growth of competing species (Rovira, 1969; Rice, 1984; Norsworthy & Meehan, 2005). Walker *et al.*, 2003; Corey and Jorge, 2008 have reported that plants release a number of low (phenolics) and high (polysaccharides, proteins) molecular weight compounds and root exudates make a significant contribution in this respect. The presence of phenolics in rhizosphere soil of *Ageratum* invaded fields indicates that these might have been released from the plants through any of the mode. Based on these observations, the growth retardatory effect of test plants may be attributed to phenolics in the rhizosphere soil of *Ageratum* invaded fields.

EXPERIMENT – 2

Objective

To study the phytotoxic effect of different parts of *Ageratum conyzoides* collected at the different stages of growth.

Hypothesis to be Tested

Since allelopathic potential varies with the growth stage of the plant and plant part, an experiment was planned to explore this and evaluate the dynamics of phytotoxicity of *A. conyzoides* at different growth stages.

Parameters Studied

Growth in terms of seedling length and seedling dry weight of *Phaseolus mungo* in response to different concentrations of extracts prepared from stem, inflorescence, roots, above-ground parts and leaves collected at different growth stages (young plantlet stage (nearly 5 cm), bud, flowering and seed stage) of the weed was studied. Besides, pH, osmotic potential and total content of phenolics of the extracts were also determined.

Methodology

Leaves, stem, above-ground parts, roots and inflorescence (wherever possible) were separately collected from the field growing *A. conyzoides* plants at different stages of its growth *viz.* plantlet stage (nearly 5 cm), bud stage, flowering and seed stage. These were dried, powdered and stored in polyethylene bags till further used. For each part of each growth stage, aqueous extracts of different concentrations i.e. 0.5, 1 and 2% were prepared. For this, twenty seeds of *Phaseolus mungo* were placed in 15 cm Petri dish lined with a thin layer of cotton and Whatman no.1 filter circle,

moistened with 8 ml of each extract or water (to serve as control). For each treatment, five replicates were maintained and arranged in a completely randomized block design. After 8 days, seedling growth in terms of length and dry weight were measured. The whole experiment was repeated. Data of mean values were analyzed by one-way ANOVA followed by DMRT and two-way ANOVA to study the possible interaction between growth stage and concentration. Besides these, values of correlation coefficient were also determined between concentration and parameter.

Results

a) Effect of extracts prepared from different parts of *A. conyzoides* at different growth stages

Seedling Length

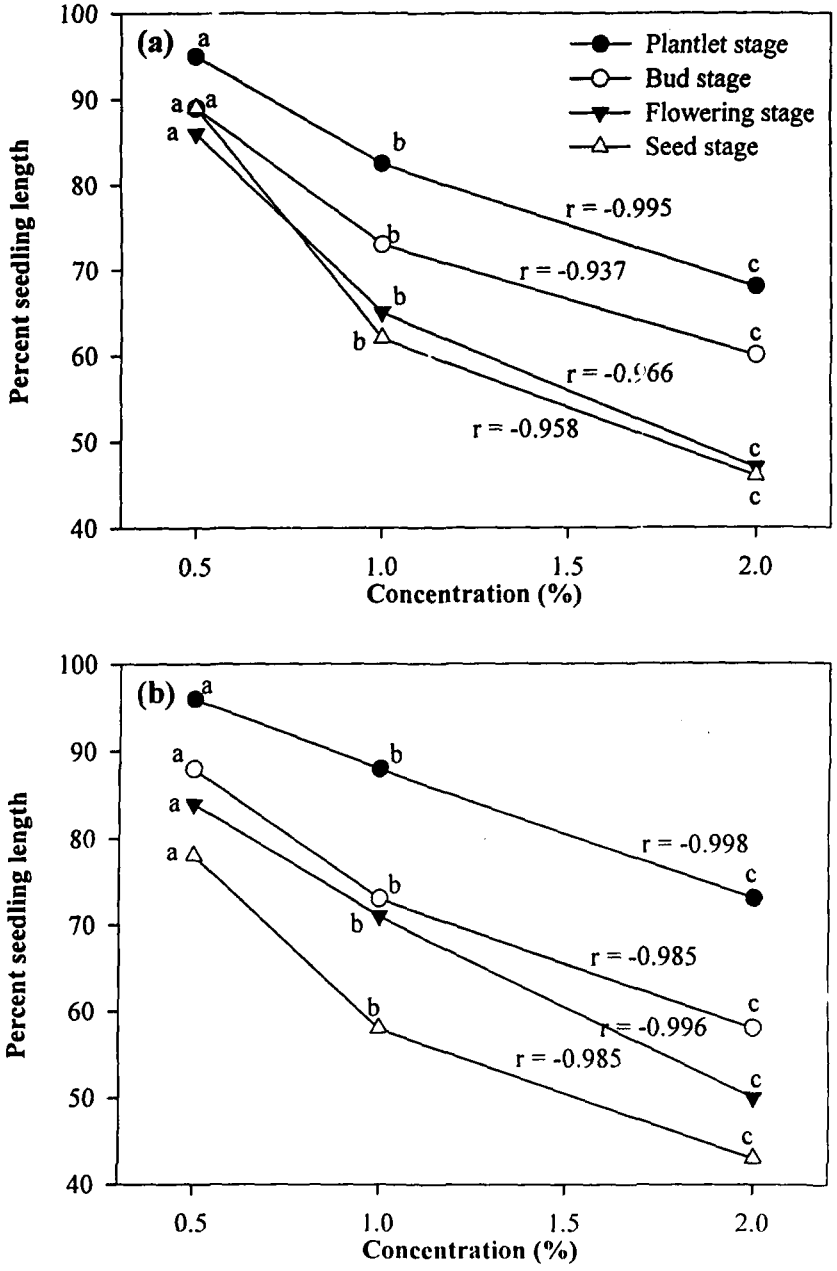
In response to extracts from above-ground parts, the seedling length of *Phaseolus mungo* decreased with the increasing concentration, irrespective of growth stage. However, maximum effect was seen in response to aqueous extracts from plants at flowering stage where seedling length was nearly 45% of that of control (16.63±1.3 cm) at highest concentration (Fig. 2.la). In extracts prepared from seed-stage almost similar effect (though slightly lesser than those of flowering stage) was observed at all concentrations. Difference wherever seen was statistically insignificant. In case of extracts prepared from bud stage of *Ageratum*, the effect was almost similar (and statistically insignificant) as that of the flowering stage. However, a significant difference was observed at highest concentration i.e. at 2%, where seedling length, was measured to be 57.3% of that of control. In all the cases, a concentration dependent response was observed. This is as also indicated by statistically significant values of correlation coefficient written against each

concentration (Fig. 2. la).

A marked decrease in the length of seedlings was observed in response to the leaf extracts also (Fig. 2.1b). Seedling length was only 37.21 % to that of control (16.84± 0.85 cm in control) with treatment of 2% leaf extract of *A. conyzoides* at flowering stage (Fig. 2.1b). In this case also, maximum reduction in seedling length was observed in response to aqueous extracts of leaves collected at flowering stage followed by the seed-stage (where at highest concentration, seedling length was 45% with respect to control). Irrespective of the stage of the plant (plantlet stage, bud, flowering and seed stage) at which extracts were prepared, a strong and reciprocal value of correlatior coefficient was observed indicating a linear relationship between concentration and effect (Fig. 2.lb).

In extracts prepared from stems, the trend of changes observed was similar to those of leaf extracts. In other words, seedling length of *P. mungo* treated with extracts was observed to be shorter compared to control and magnitude of inhibition was more with increasing concentration of extracts (irrespective of the stage of the plant). In this case also, greater reduction of seedling length of *P. mungo* was observed with stem extracts at flowering stage (seedling length in control is 17.5±0.66 cm). This was followed by those of seed-stage extracts. The only exception observed was effect of stem extract at 0.5 and 1% concentration collected from seed and bud stage. The effect of vegetative stage was more compared to seed stage at lower concentrations. The values were statistically significant at *5%* but insignificant at 1 % (Fig. 2.2a).

Fig. 2.1. Effect of aqueous extracts of (a) above-ground parts and (b) leaves of *A. conyzoides* collected at different growth stages on seedling length of *P. mungo*.

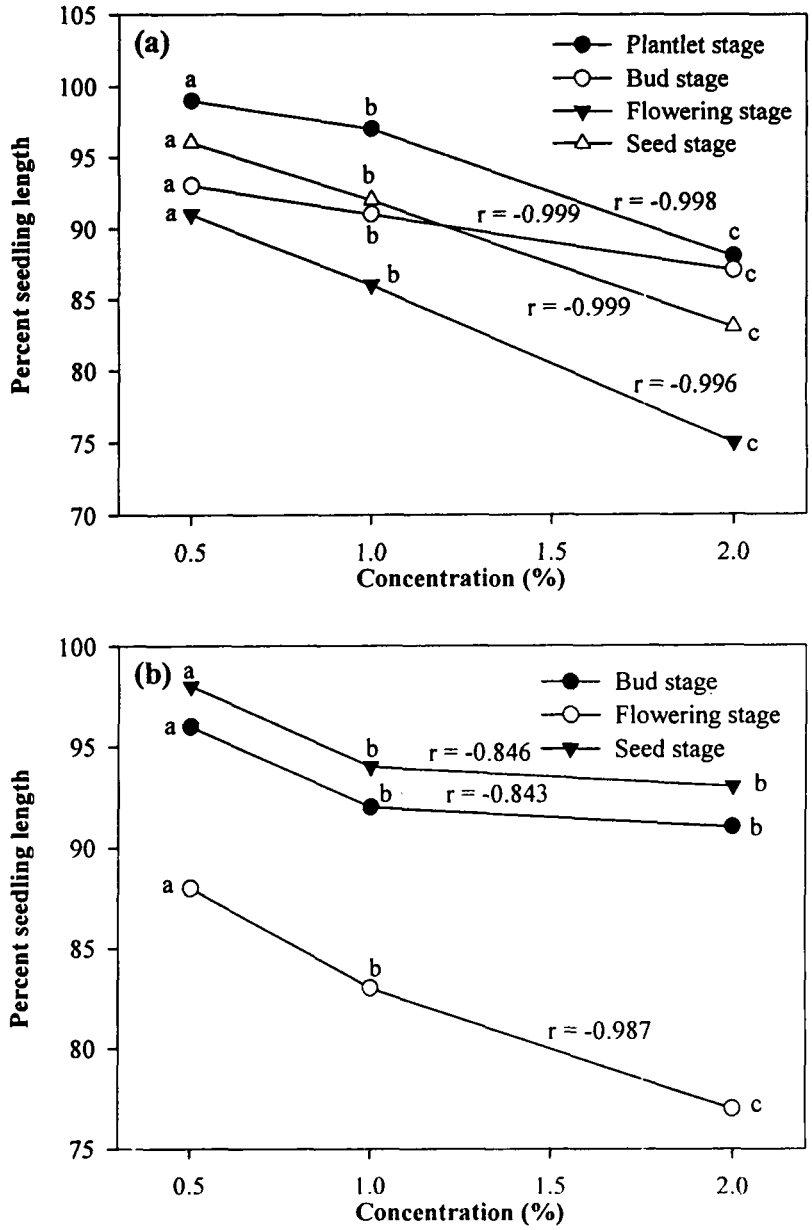


Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Likewise, inflorescence extracts were prepared from plants at three stages i.e. pre-flowering or bud stage (transition stage), flowering stage and seed stage. Compared to control, irrespective of the concentration, more reduction was

observed at flowering stage followed by bud and seed stage. With the treatment of

Fig. 2.2. Effect of aqueous extracts of (a) stem and (b) inflorescence of *A. conyzoides* collected at different growth stages on seedling length of *P. mungo*.

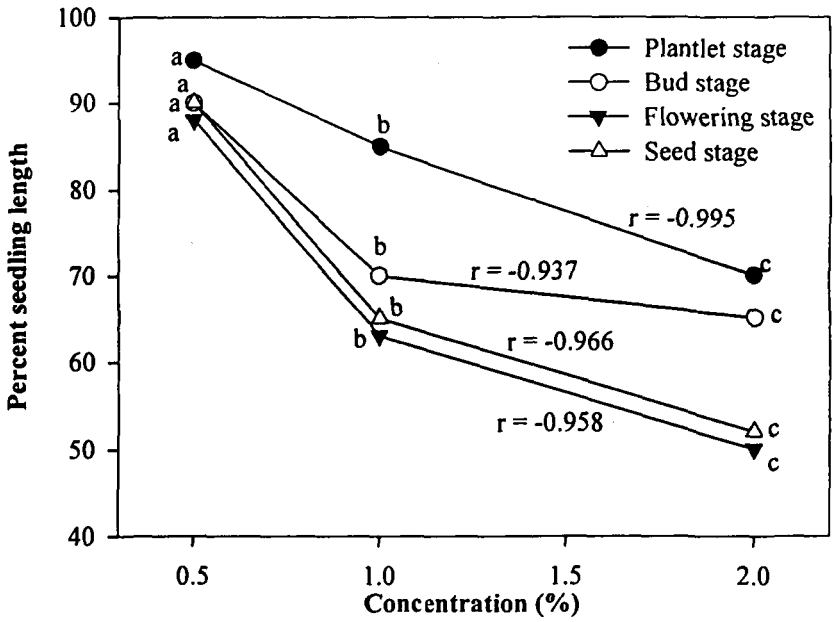


Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

inflorescence extracts prepared from bud and seed stage, very little reduction in seedling length was observed. However, in earlier case, the value of correlation coefficient was statistically significant, reciprocal and strong (Fig. 2.2b). Seedling

length in response to 2% root extracts from flowering stage was nearly 43% of that in water treated control where the average seedling length was 16.94±0.92 cm. At this concentration, maximum reduction of seedling length was observed at flowering stage followed by seed stage, bud stage and plantlet stage (Fig. 2.3). The values of correlation coefficient, here also, were more than 0.9 and reciprocal indicating concentration dependent response in case of each type of the extract.

Fig. 2.3. Effect of aqueous extracts of roots of *A. conyzoides* collected at different growth stages on seedling length of *P. mungo*.



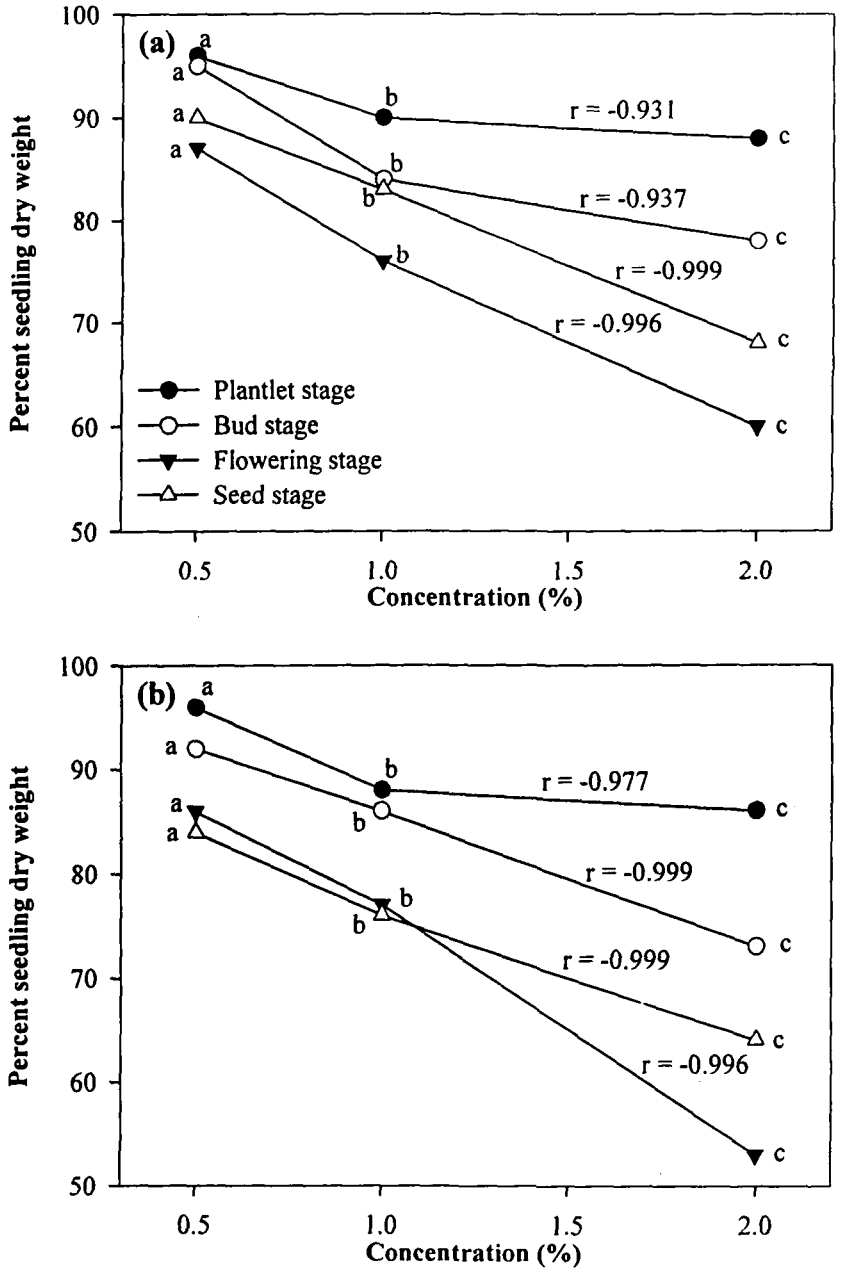
Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Seedling Dry Weight

In general, dry weight of 8-day-old seedling of *P. mungo* was estimated to be 22.2±1.55, 23.04±0.81, 21.02±0.92, 22.74±0.94 and 20.97±0.84 mg in control of above-ground parts, leaves, stem, inflorescence and in root extracts, respectively. Like seedling length, a similar trend was observed in dry weight with extracts of above ground parts, leaves, stem, inflorescence and roots (Figs. 2.4a,b, 2.5a,b, 2.6). With the

treatment of extracts prepared from above ground parts, leaves, stems, inflorescence

Fig. 2.4. Effect of aqueous extracts of (a) above-ground parts and (b) leaves of *A. conyzoides* collected at different growth stages on seedling dry weight of *P. mungo*.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

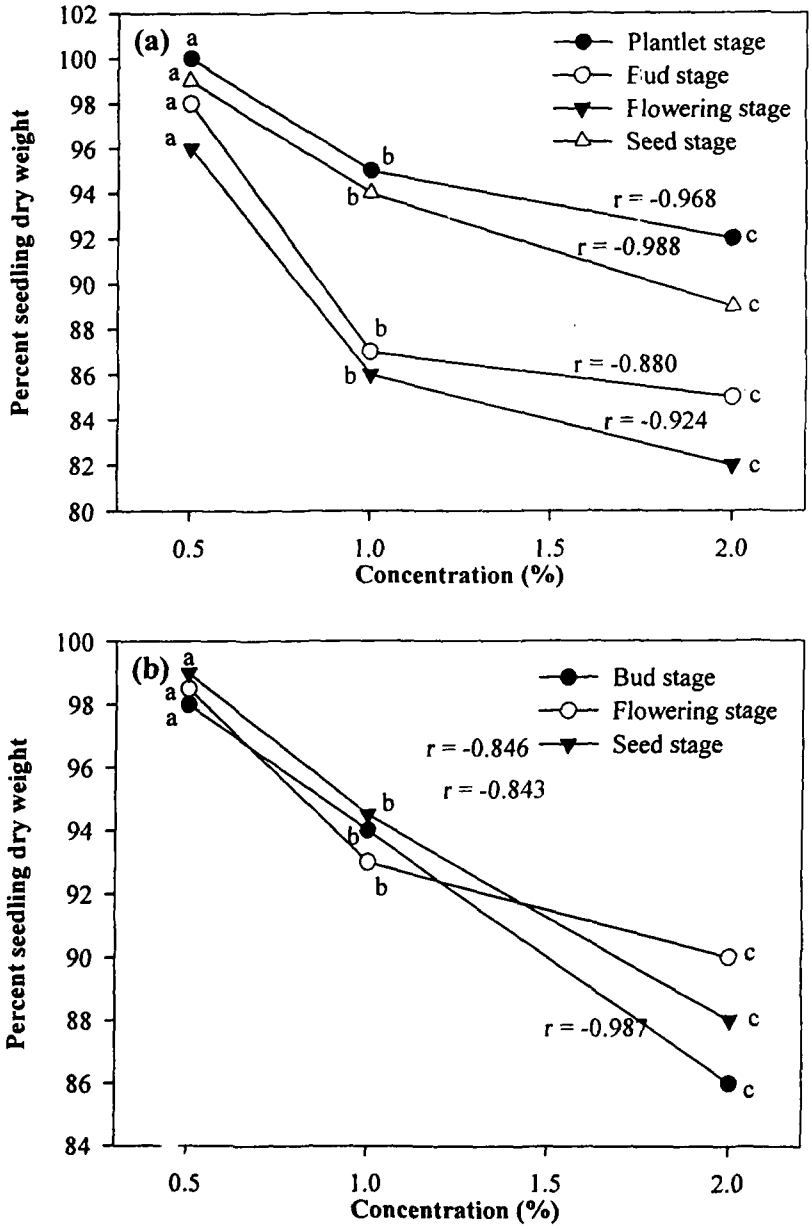
or roots, a statistically significant difference in dry weight was observed (irrespective of the growth stage of *Ageratum*; Fig. 2.4a,b, 2.5a,b, 2.6, respectively). At lowest

concentration (0.5%), particularly in case of inflorescence or root extracts, the seedling dry weight of *P. mungo* was almost the same. The difference among extracts prepared from plants at different growth stages was statistically insignificant.

b) Comparison of phytotoxicity of different extracts prepared from different parts at flowering stage

A comparison of the impact of extracts made from different parts of *Ageratum* at flowering stage (at flowering stage, maximum reduction in seedling length and seedling dry weight was observed as already explained above) was also made. It was observed that in response to 2% leaf extracts, over 60% of reduction in seedling length was observed compared to control (Fig. 2.7a). It was followed by extracts of roots and above ground parts where the seedling length of *P. mungo* did not differ much at 1% concentration. In fact, in 2% extracts, seedling length was reduced more in response to leaf extracts than root extracts. At the lowest concentration (0.5%) no change in seedling length was observed in case of roots and leaf extracts (Fig. 2.7a). A similar level of phytotoxicity was observed with extracts from above-ground parts where in response to 0.5% extracts seedling length was nearly 86.8% of control. However, seedling length in response to inflorescence and stems extracts did not differ much and was nearly 76% of control at 2% concentration. Almost similar trend of changes were observed in case of seedling dry weight also (Fig. 2.7b). The decreasing order of phytotoxicity was therefore observed as Leaves > Roots > Above-Ground Parts > Stems > Inflorescence.

Fig. 2.5. Effect of aqueous extracts of (a) stem and (b) inflorescence of *A. conyzoides* collected at different growth stages on seedling dry weight of *P. mungo*.



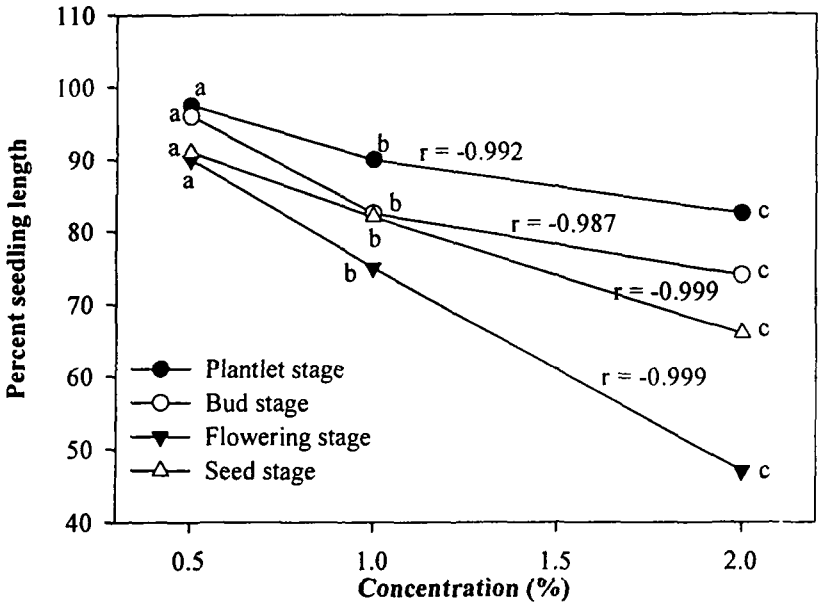
Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

c) Interaction between plants parts and different growth stages of *A. conyzoides*

Since, the study was conducted to test the comparative phytotoxicity of different parts collected from plants at different growth stages and also at different

concentrations, the data were also analyzed by two way ANOVA in order to find out the interaction between different concentration of extracts of each part with respect to

Fig. 2.6. Effect of aqueous extracts of prepared from collected at different growth stages on the seedling dry weight of *P. mungo.*



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

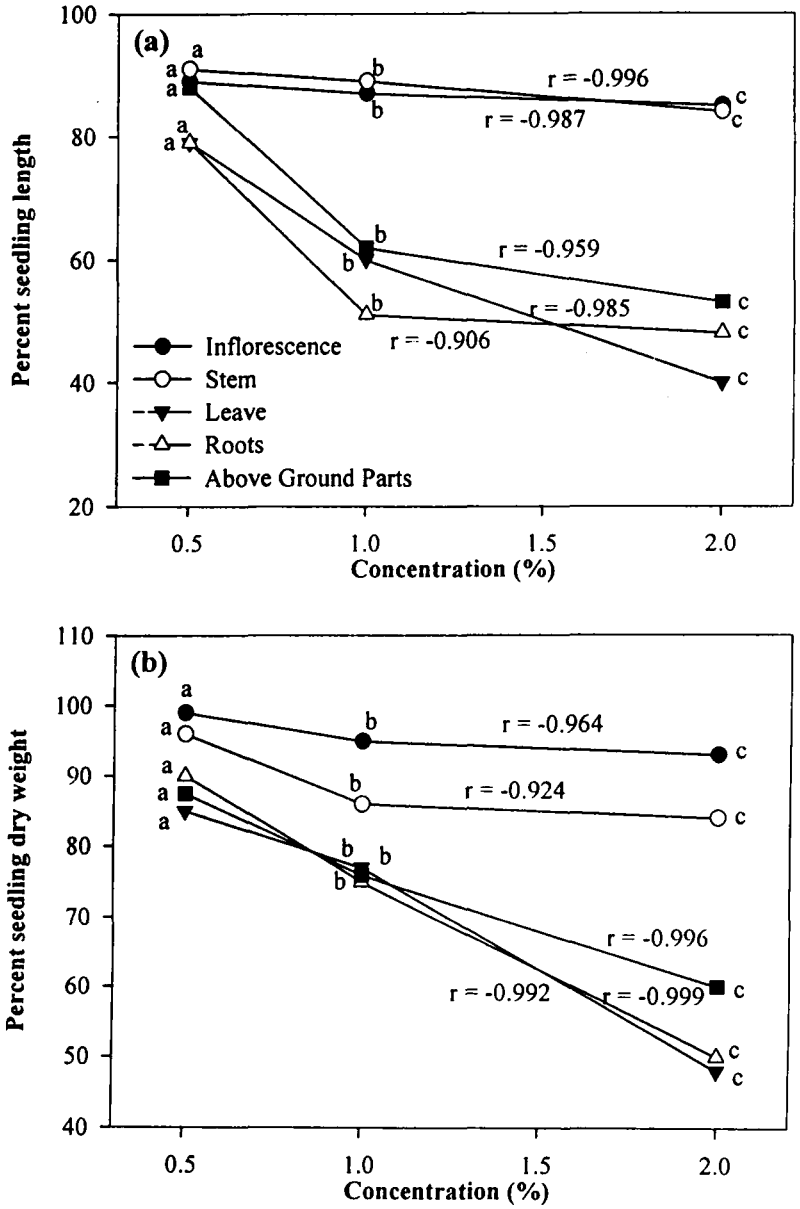
respective growth stage. The analysis was conducted both in case of seedling length and seedling dry weight. Though concentration of extracts of each part and growth stage of plant had a significant effect on seedling length of the test plant (except stems *v/s* growth stage, that was insignificant, Table 2.1), yet, there was little interaction between these two i.e. concentration and stage as revealed by insignificant *f*-values (Table 2.1).

However, roots were only exception where both concentration and stage collectively affected the seedling length.

In case of seedling dry weight, concentration had a significant effect on seedling parameter as revealed by all significant values with respect to concentration

of each part (Table 2.2). Stage of plant, however, had a significant influence on seedling dry weight only with respect to roots, leaves and above-ground parts. The effect was statistically significant only in case of leaves and roots. In rest of the treatments this was insignificant (Table 2.2).

Fig. 2.7. Effect of different concentrations of extracts of different parts of *A. conyzoides* plants at flowering stage on the (a) seedling length and (b) dry weight of *P. mungo*.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Table 2.1 Relationship between plant stage and concentration of extracts of different parts *A. conyzoides* and its effect on seedling length analyzed by two way ANOVA.

Parts of the plant	F-value (stage of the plant)	F-value (concentration)	F-value (growth stage × concentration)
Inflorescence	29.88*	28.76*	3.99ns
Stems	5.87ns	38.59*	1.01ns
Leaves	29.22*	224.7*	4.47ns
Roots	23.75*	182.93*	3.814*
Above ground parts	9.48*	160.93*	2.059ns

* indicates significant from control at $P<0.05$; ns indicates insignificant.

Table 2.2 Relationship between plant stage and concentration of extracts of different parts *A. conyzoides* and its effect on seedling dry weight analysed by two way ANOVA.

Parts of the plant	F-value (stage of the plant)	F-value (concentration)	F-value (stage × concentraton)
Buds/Inflorescence	0.15ns	22.85*	0.2ns
Stems	646ns	46.26*	1.26ns
Leaves	39.10*	261.44*	9.55*
Roots	22.07*	226.39*	5.14*
Above ground parts	12.14*	82.63*	2.73ns

* indicates significant from control at $P<0.05$; ns indicates insignificant.

Table 2.3 values of pH, osmotic potential and total phenolic content in different concentrations of extracts of inflorescence collected during different stages of *A. conyzoides*.

Parameters	Extract conc. (%)	Bud stage	Flowering stage	Seed stage
pH	0.5	6.9^a	6.86^a	6.87^a
	1.0	6.84^a	6.84^a	6.80^a
	2.0	6.80^a	6.80^a	6.75^a
Osmotic potential (-bars)	0.5	0.22^a	0.30^a	0.26^a
	1.0	0.26^a	0.36^a	0.27^a
	2.0	0.30^a	0.48^a	0.28^a
Total phenolic content (µg/ml)	0.5	30.03^c	48.4^c	42.4^b
	1.0	40.4^b	60.5^b	50.2^b
	2.0	54.6^a	124.33^a	60.0^a

Different alphabets within a parameter in a column represent significant difference at $P<0.05$.

In order to explore the possible reasons for observed growth inhibition effects, a few more studies *viz.* determination of pH, osmotic potential and amount of phenolics, were undertaken. The study didn't reveal much change in the pH of extracts, though it differed within different extract concentrations. It varied from 6.38 to 6.96. For inflorescence, stem, root and above-ground parts, the difference in the pH of different extracts, (at different growth stages) was statistically insignificant (Table 2.3, 2.4, 2.6, 2.7, respectively). However, pH of leaf extracts at flowering and seed stage decreased significantly at 2% concentration compared to 0.5 and 1% (Table 2.5).

Table 2.4 values of pH, osmotic potential and content of total phenolics in different concentrations of extracts of stem collected during different stages of *A. conyzoides*.

Parameters	Extract conc. (%)	Plantlet stage	Bud stage	Flowering stage	Seed stage
pH	0.5	6.88^a	6.83^a	6.81^a	6.85^a
	1.0	6.81^a	6.75^a	6.70^a	6.78^a
	2.0	6.75^a	6.69^a	6.66^a	6.68^a
Osmotic potential (-bars)	0.5	0.18^a	0.26^a	0.34^a	0.30^a
	1.0	0.22^a	0.34^a	0.37^a	0.32^a
	2.0	0.26^a	0.38^a	0.44^a	0.39^a
Total phenolic content (µg/ml)	0.5	28.18^c	35.25^c	45.45^b	40.0^c
	1.0	42.04^b	54.4^b	82.4^b	50.88^b
	2.0	58.3^a	69.9^a	100.5^a	62.4^a

Different alphabets within a parameter in a column represent significant difference at $P<0.05$.

Further, the osmotic potential (OP) of extract was also measured. In leaf extracts, values of OP ranged from -0.31 bars to -0.94 bars (Table 2.5). In extracts of different stages (except plantlet stage), the osmotic potential increased with concentration (Table 2.5). The amount of phenolics was also determined in extracts from different parts at various growth stages. In leaf extracts, amount of phenolics

was about 858 µg/ml in 2% extracts, from leaves at the flowering stage. Among different parts, content of phenolics was the maximum in leaves and least in inflorescence.

Table 2.5 values of pH, osmotic potential and content of total phenolics in different concentrations of extracts from leaves collected from *A. conyzoides* at different stages.

Parameters	Extract conc. (%)	Plantlet stage	Bud stage	Flowering stage	Seed stage
pH	0.5	6.83^a	6.75^a	6.68^a	6.78^a
	1.0	6.77^a	6.69^a	6.50^a	6.66^a
	2.0	6.66^a	6.52^a	6.38^b	6.49^b
Osmotic potential (-bars)	0.5	0.31^a	0.33^b	0.39^b	0.32^b
	1.0	0.31^a	0.68^a	0.80^a	0.69^a
	2.0	0.33^a	0.76^a	0.94^a	0.82^a
Total phenolic content (µg/ml)	0.5	157.91^c	209.92^c	288.89^c	321.92^c
	1.0	219.18^b	446.85^b	467.22^b	429.22^b
	2.0	530.7^a	858.0^a	783.64^a	813.04^a

Different alphabets within respective parameter in a column represent significant difference at $P<0.05$.

Table 2.6 values of pH, osmotic potential and content of total phenolics in different concentrations of extracts of roots collected during different stages of *A. conyzoides*.

Parameters	Extract conc. (%)	Plantlet stage	Bud stage	Flowering stage	Seed stage
pH	0.5	6.96^a	6.75^a	6.75^a	6.73^a
	1.0	6.86^a	6.72^a	6.62^a	6.64^a
	2.0	6.82^a	6.66^a	6.50^a	6.60^a
Osmotic potential (-bars)	0.5	0.12^b	0.14^b	0.19^b	0.23^b
	1.0	0.27^b	0.34^b	0.37^b	0.64^a
	2.0	0.50^a	0.72^a	0.92^a	0.80^a
Total phenolic content (µg/ml)	0.5	30.06^c	80.52^c	123.1^c	90.58^c
	1.0	68.63^b	191.61^b	249.36^b	224.76^b
	2.0	188.55^a	312.15^a	508.7^a	341.61^a

Table 2.7 values of pH, osmotic potential and content of total phenolics in different concentrations of extracts of above-ground parts collected during different stages of *A. conyzoides*.

Parameters	Extract conc. (%)	Plantlet stage	Bud stage	Flowering stage	Seed stage
pH	0.5	6.82[a]	6.79[a]	6.79[a]	6.88[a]
	1.0	6.70[a]	6.69[a]	6.68[a]	6.78[a]
	2.0	6.54[a]	6.60[a]	6.58[a]	6.72[a]
Osmotic potential (-bars)	0.5	0.30[a]	0.32[a]	0.33[a]	0.26[b]
	1.0	0.51[a]	0.59[a]	0.64[a]	0.46[b]
	2.0	0.67[a]	0.78[a]	0.86[a]	0.82[a]
Total phenolic content (µg/ml)	0.5	78.18[c]	135.86[c]	169.28[c]	180.93[c]
	1.0	190.23[b]	203.63[b]	284.53[b]	239.84[b]
	2.0	205.35[a]	381.84[a]	475.60[a]	310.38[a]

Different alphabets within a parameter in a column represent significant difference at $P<0.05$.

Discussion

It is clear from the present study that different parts of the weed *A. conyzoides* exhibited phytotoxic potential through their aqueous extracts, though the magnitude of phytotoxicity varied with plant part and growth stage. The study, therefore, indicated that some growth inhibitors are present in the extract that might be affecting the growth of the test plant - *Phaseolus mungo.* A number of studies have indicated that aqueous extracts of weeds, particularly the exotic weeds, are phytotoxic in nature and thus reduce the growth of other plants (Qasem and Foy, 2001). Some recent studies indicating the phytotoxic / allelopathic effect of aqueous extracts of weeds include *Mikania micrantha* (Ismail and Kumar, 1996), *Vulpia* sp, (An *et al.*, 1999), *Cyperus rotundus* (Quayyum *et al.*, 2000), *Cardaria draba* (Kiemnec and Mclnnis, 2002), *Parthenium hysterophorus* (Batish *et al.*, 2002a; Singh *et al.*, 2003a), *Brassica nigra* (Tawaha and Turk, 2003), *Raphanus raphanistrun* (Norsworthy, 2003), and

Ageratum conyzoides (Batish *et al.*, 2002b; Singh *et al.*, 2003b,c), *Parthenium hysterophorous* (Javaid *et al.*, 2006), *Tagetes minuta* and *Eupatorium rugosum* (Jihyon & Kewcheol 2006). All these studies indicate the release of phytotoxic chemicals during the preparation of aqueous extracts. Under natural conditions, metabolites including organic acids, minerals, carbohydrates and amino acids are released or leached from various parts of plant including foliage, stem, flowers and fruits of the plant by forming aqueous solution with rainwater, dew water and mist (Tukey and Morgan, 1964). Through this process, a number of phytotoxic chemicals are also leached that brings about inhibitory effects on the other plant (Rice, 1984). The phenomenon of leachation is wide spread in nature and plays both stimulatory and inhibitory roles and is thus ecologically important (Tukey and Mecklenburg, 1964). However, the reports regarding the inhibitory effects of leachation are more common (Rice, 1984).

The observed differential phytotoxicity of *A. conyzoides* may be attributed to the presence of variable amounts of phytotoxic substances in different parts that leach out under natural conditions. Foliar leachates have been regarded to be most phytotoxic in nature (Xuan *et al.*, 2004) probably owing to their proportionately greater biomass and with greater metabolic activity or production of more metabolites (Xuan *et al.*, 2004). In *A. conyzoides* also, more phytotoxicity and phenolics were found in leaves.

Studies have also indicated that phytotoxicity of leachable allelochemicals is dependent upon several factors such as concentration, flux rate, age and metabolic stage of part and environmental conditions (Rice, 1984; Wyman-Simpson *et al.*, 1991; Wardle *et al.*, 1993; Weidenhamer, 1996; Kong *et al.*, 2008). In the present study, it

was noticed that not only the allelopathic activity of the weed changes with plant part but also, with stage of growth. This is an important observation that can be utilized for minimizing the phytotoxicity of the weed during its heavy infestation in the croplands. Several workers have reported that plants at reproductive stage or flowering stage are more phytotoxic than any other stage (El-Khatib, 1998; Mandal, 2001).

Generally, in studies with aqueous extracts, the observed inhibitory effect are attributed to changes in pH and osmotic potential thereby raising concerns about allelopathy and its ecological existence and relevance (Harper, 1977; Keeley, 1988; Conway *et al.,* 2002). In the present study, pH of extracts ranged from 6.38 to 6.96, which is considered optimum for plant growth (Macias *et al.,* 2000). Likewise, the osmotic potential range (-0.12 bars to -0.94 bars) is again unlikely to cause any inhibitory effect on the plant growth (Mersie and Singh, 1987). After making these observations, it could be concluded that extract might possess growth inhibitory metabolites that leach out in water. In order to find out nature of these growth inhibiting substances, the amount of total phenolics in extracts was determined as these are most common water soluble group of allelochemicals playing an important role in allelopathy (Rice, 1984; Appel, 1993; Mizutani, 1999; Chihua *et al.,* 2007; Sisodia and Siddiqui, 2008). An appreciable amount of phenolics was determined in all the extracts and their amount increased with extract concentrations. Further, the amount of phenolics also correlated with phytotoxic effect of the weed part (although no correlation analysis was made, it is apparent from the data that phytotoxic effect was more where the amount of phenolics was also more).

Therefore, on the basis of these observations following conclusions can be made:

- Different parts of weed. *A. conyzoides* exhibit differential phytotoxicity and the degree of phytotoxicity with respect to plant part was in the order:

  Leaves > Roots > Above-Ground Parts > Stems > Inflorescence

- Leaves being more in biomass per plant contribute relatively more towards phytotoxicity compared to other parts of the plant.
- Phytotoxicity or allelopathy of different parts was the maximum at flowering stage followed by seed-stage and bud stage. It was the minimum at the plantlet stage.
- Presence of phenolics imparted the allelopathic / phytotoxic property to the different parts as evidenced from their amount and degree of inhibition of test plant.

EXPERIMENT – 3

Objective

To study the impact of above-ground parts of *Ageratum conyzoides* (flowering stage) on growth performance of some test plants and changes in soil properties when added to the soil.

Hypothesis to be Tested

The plants of *A. conyzoides* grow abundantly in agricultural fields. These are usually cut and thrown as such in the same area. Upon drying or decomposition these mix with soil and may interfere with the growth and development of plants. To test this hypothesis, the present experiment was planned with above-ground parts either amended directly in the soil or in the form of its aqueous extracts.

Parameters studied

Effect of above-ground parts of *A. conyzoides* was studied by incorporating the soil with aqueous extracts or residues and determining the effect on growth of plants namely *Anagalis arvensis*, *Brassica oleracea* var. *botrytis*, *Cicer arietinum*, *Phaseolus mungo* and *Oryza sativa* with respect to their seedling lengths and dry weights.

Further, changes in some soil properties like pH, conductivity, osmotic potential (OP), amount of total phenolics, organic carbon (OC), organic matter (OM) and amount of macro and micro nutrients i.e. N, P, K, Ca, Mg, Na and Cl, HCO_3, Zn, Cu, Mn, Fe were also determined in amended and unamended soils.

Methodology

Above-ground parts of *A. conyzoides* (collected at flowering stage from

agricultural fields and other areas around Aligarh) were separated from roots. These were shade dried, powdered and stored in polyethylene bags for further use. Aqueous extracts in the concentration of 0.5, 1, 2 and 4% (w/v) were prepared with pure water. Their effects were studied on growth of different plants under laboratory conditions as per details discussed in chapter material and methods. Likewise, growth studies were also performed with extracts and above-ground powdered material (or hereafter knowing residue) amended in soil simulating natural conditions. For this, extracts of each concentration (500 ml/kg soil) and above-ground powdered material (0.5, 1, 2 and 4 g/100g soil; w/w) were mixed into the soil. For each treatment, five replicates were maintained and in each set, five seeds of above mentioned plants were grown. For comparison, one set of five replicates was also maintained where neither extract nor the residue was added to the soil to serve as control. In each set, both under laboratory conditions or under natural conditions, growth of seedlings in terms of length and dry weight was measured after eight days. In order to find out the reasons of reduction of growth, extracts as well as amended soils were subjected to some physico-chemical analysis. Besides this, amended as well as unamended soils were also subjected to nutrient (macro as well as micro nutrients) analysis, following the procedures discussed in chapter material and methods. All the experiments were repeated and mean values of data were analyzed with one-way ANOVA.

Results

a) Growth Studies under Laboratory Conditions

The extracts prepared from above-ground parts of *A. conyzoides* significantly affected the seedling length of all the five test plants namely *Anagalis arvensis*, *Brassica oleracea* var. *botrytis*, *Cicer arietinum*, *Phaseolus mungo*, *Oryza sativa*

compared to control. (Fig.3.la).The seedling length and seedling dry weight were seen to decrease with increasing concentration of extracts in all the test plants. Among the test plants, maximum effect was seen in case of *C. arietinum.* In this none of the seeds germinated in response to 4% extract solution of *A. conyzoides* (Fig. 3.1a,b).

At 4% concentration, nearly 70% reduction in seedling length of *Anagalis arvensis* was noticed. In *Brassica oleracea* var. *botrytis* seedling length was measured to be 16.6±1.3 cm in control, whereas with the treatment of extracts, seedling length decreased significantly at every concentration. In this case, nearly 83% reduction in seedling length to that of control was seen (Fig. 3.la).

In *C. arietinum*, where average seedling length measured to be 17.02±0.18 cm in control, 100% inhibition was observed at 4% concentration (here none of the seeds germinated). Even at 2% concentration, the reduction was too high (nearly 72%; Fig. 3.1 a). Similar observations were made with respect to *P. mungo* and *O. sativa* where the lengths of seedling in control were 17.6±1.1 and 9.8±0.31 cm, respectively. These decreased with increasing concentrations of aqueous extracts (Fig 3.1 a). Seedling length of *P. mungo* and *O. sativa* treated with 4% extract treatment was measured to be 4.2±0.65 and 0.77±0.21 cm thus exhibiting a reduction of nearly 76% and 95% respectively. It is clear from the figure (3.la) that values of correlation coefficient were significant when curves were drawn between concentration of extract and seedling length of different test plants. With respect to all plants, the values of *r* were greater than -0.9, thus, exhibiting a strong and reciprocal correlation.

Not only the seedling length, even dry biomass accumulation in seedlings of different test plants was observed to be less in response to any of the concentrations of the extracts (Fig. 3.1b). In *Anagalis arvensis* dry weight per seedling was 6.25±0.3

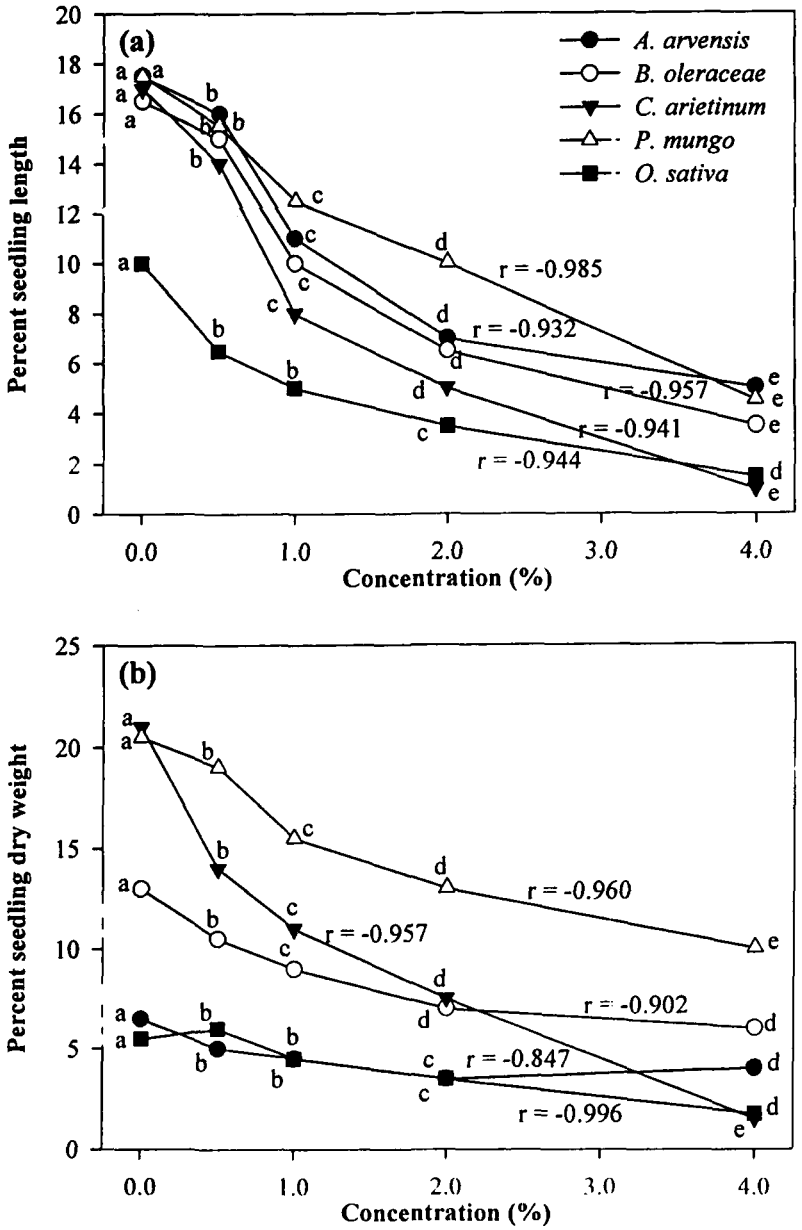
mg and nearly 53% reduction was observed at the highest concentration of extract used. In *Brassica oleracea* var. *botrytis* almost similar trend was observed and the dry weight was measured to be 5.68±1.8 at 4% extract concentration compared to I2.73±2.5 mg in control. In *C. arietinum*, the dry weight of seedling in control was 21.07±1.9 mg. At 1 and *2%* concentration of extract the dry weight was measured to be 10.69±0.08 mg and 7.41±0.29 mg, respectively, exhibiting a reduction of nearly 50 and 65%. With the treatment of at 4% extracts, a complete inhibition of germination was observed. In *P. mungo* also, the dry weight decreased with the increasing concentration of extract and thus biomass of seedlings at highest concentration was 9.21±1.3 mg compared to 20.26±1.0 mg in control, indicating a reduction of about 55%. In *O. sativa,* where seedling dry weight was measured to be 5.65±0.33 mg in control, a similar trend of reduction was observed with respect to the treatment of different concentrations of *Ageratum* extracts (Fig. 3.1b).

In dry weight also, values of correlation coefficient were calculated to be strong and reciprocal (> 0.9, except *Anagalis arvensis* where it was -0.84). Among the five test plants, maximum growth retardatory effect was observed in *C. arietinum* followed in sequence by *O. sativa*, *Brassica oleracea* var. *botrytis*, *P. mungo* and *Anagalis arvensis* respectively (Fig. 3.1b).

(b) Growth Studies in Experimental Dome

Since the extract alone retarded the growth of all test plants, these were amended into soil so as to determine their effect on the growth of test plants in soil medium. In this case also, the growth of all test plants was significantly reduced compared to control though the magnitude of reduction was lesser than in extract alone (Fig. 3.2a, b). In *Anagalis arvensis* the average seedling length in control was

Fig. 3.1. Effect of different concentrations of extracts prepared from above ground parts of *A. conyzoides* on (a) seedling length and (b) seedling dry weight of test plants.

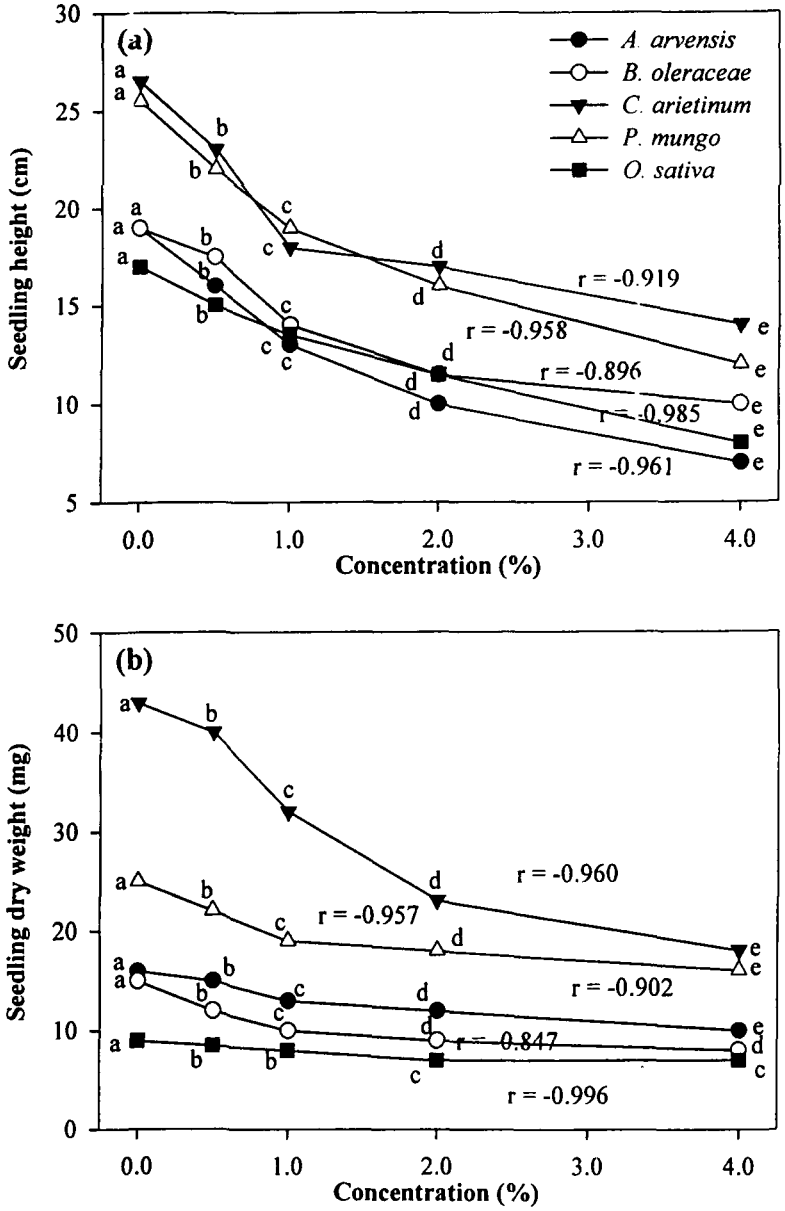


Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

18.76±0.4cm. It decreased significantly in all the extract amended soils. Thus a reduction by 66% to that of control was noticed in case of supplement with 4% extract (Fig. 3.2a). In rest of the plants *viz. Brassica oleracea* var. *botrytis*, *C. arietinum*, *P. mungo and O. sativa*, grown in extract amended soil, almost similar

trend of growth reduction was observed in the extract amended soil (Fig 3.2a).

Fig. 3.2. Seedling length (a) and dry weight (b) of test plants grown in soil amended with different concentrations of extracts prepared from above ground parts of *A. conyzoides*.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

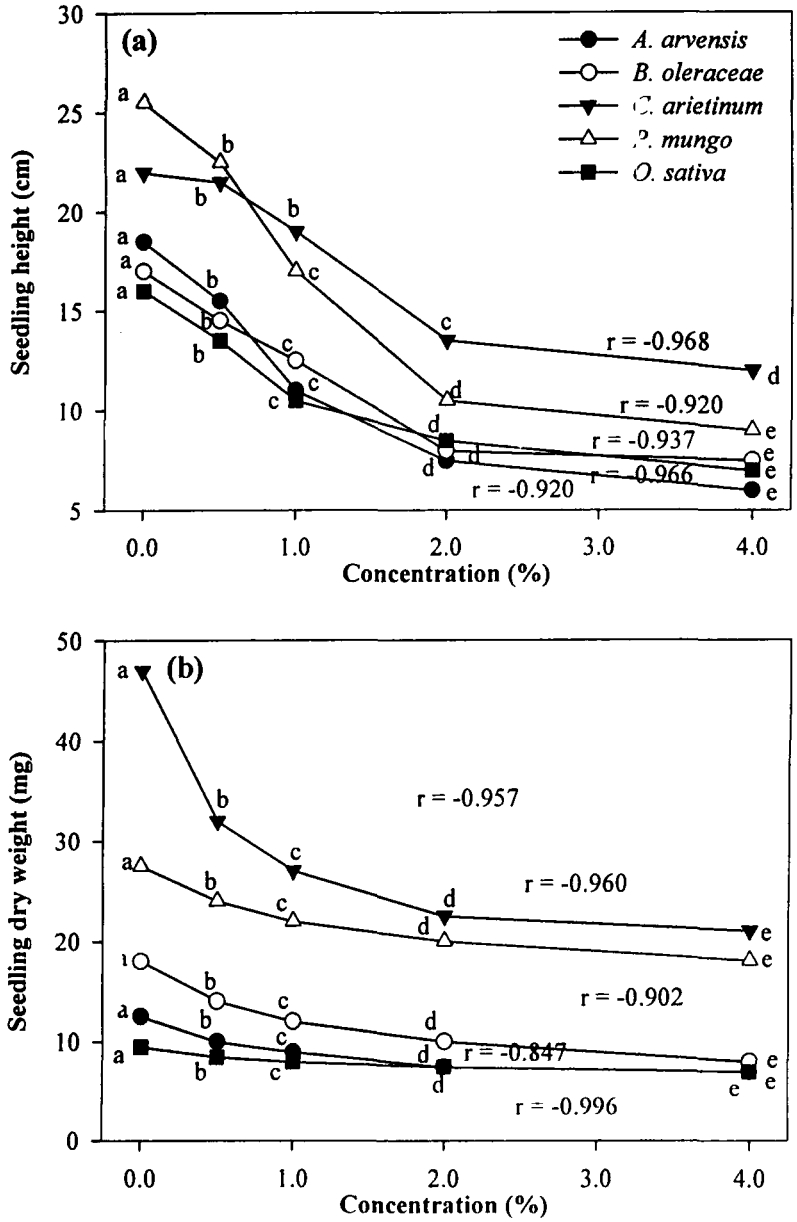
In addition to reduced length, the dry weight of plants was also seen reduced significantly compared to control. In all plants reduction in soil amended with 0.5% extract was though less, yet statistically significant compared to control. At higher concentrations (2 or 4%) however, the decrease in seedling length and biomass was

appreciable. Maximum reduction in such cases was observed in *O. sativa* and *C. arietinum* compared to other test plants (Fig. 3.2b). The correlation coefficient between concentration of extract and seedling length or seedling dry weight was also strong and reciprocal. Test plants grown in soils amended with above ground powdered material of *A. conyzoides* also exhibited lesser growth compared to control. Both, seedling length and dry weight were significantly reduced. In response to amendment of 4% above ground parts of *A. conyzoides*, retardation was observed to vary from 55% (in *C. arietinum*) to 70% (in *O. sativa*; Fig. 3.3a).

Likewise, the dry biomass was also induced in similar manner. However, the reduction varied from 45-70%, when grown in soil amended with 4 g powder (Fig. 3.3b). A strong reciprocal correlation coefficient with value > -0.9 was observed in almost all the cases (Fig. 3.3 a,b). In other words, it is clear that growth of test plants was not only reduced in the medium of extracts but also in soil amended with powder or its extracts. In order to find out the reasons for the observed growth inhibition, the aqueous extracts as well as amended soils with extracts and powder were analyzed for pH, osmotic potential and amount of total phenolics. In case of extracts, pH values ranged from 6.89 to 6.4 in its concentrations varying from 0.5 to 4% (Table 3.1).

Osmotic potential of extract in concentration 0.5 to 4% ranged from -0.33 bars to-1.04 bars. The amount of total phenolics was found to be 518.8±7.2 μ/ml in 4% extract. Even in the lowest concentration (0.5%), amount of phenolics was 258.7±1.6 μ/ml. Likewise, the various soils amended with either extracts or residues of *A. conyzoides* were also analyzed for pH and conductivity. The pH in the amended soils was found to be slightly lesser than that in unamended soil. In the powder amended soils, it ranged from 7.5 to 7.26 indicating slight (statistically significant)

Fig. 3.3. Seedling length (a) and dry weight (b) of test plants grown in soil amended with powder of above ground parts of *A. conyzoides*.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

decrease (Table 3.2). Likewise, in the extract-amended soils, very little change in pH values was observed; the differences were statistically insignificant (at lower concentrations). Conductivity on the other hand, increased significantly in the amended soil (Table 3.2).

Table 3.1 Conductivity, pH, osmotic potential and total phenolic content of extracts of above-ground parts of *A. conyzoides*.

Property	Concentrations (%)			
	0.5	1.0	2.0	4.0
pH	6.89 ± 0.04^{a}	6.64 ± 0.02^{b}	6.55 ± 0.08^{c}	6.40 ± 0.05^{d}
Conductivity (mS)	0.923 ± 3.46^{d}	1.636 ± 3.46^{c}	2.33 ± 0.05^{b}	2.89 ± 0.04^{a}
Osmotic potential (-bars)	0.33 ± 0.02^{d}	0.59 ± 0.04^{c}	0.84 ± 0.03^{b}	1.04 ± 0.04^{a}
Total phenolic content (µg/ml)	258.7 ± 1.55^{d}	400.2 ± 1.93^{c}	444.72 ± 1.48^{b}	518.8 ± 7.2^{a}

Different superscripts within a parameter in a row represent significant difference at $P<0.05$.

In both extract and powder amended soil, percent organic carbon increased significantly compared to control. However, the magnitude of increase was more in powder amended soil (Fig. 3.4a). At 4% concentration of amendment, it increased by nearly 3.2 and 2.8 times over control in powder and extract amended soils, respectively (Fig. 3.4a). Almost similar trend of increase was observed in organic matter content.

Table 3.2 Changes in pH and conductivity of soils amended with powder or extracts of above-ground parts of *A. conyzoides*.

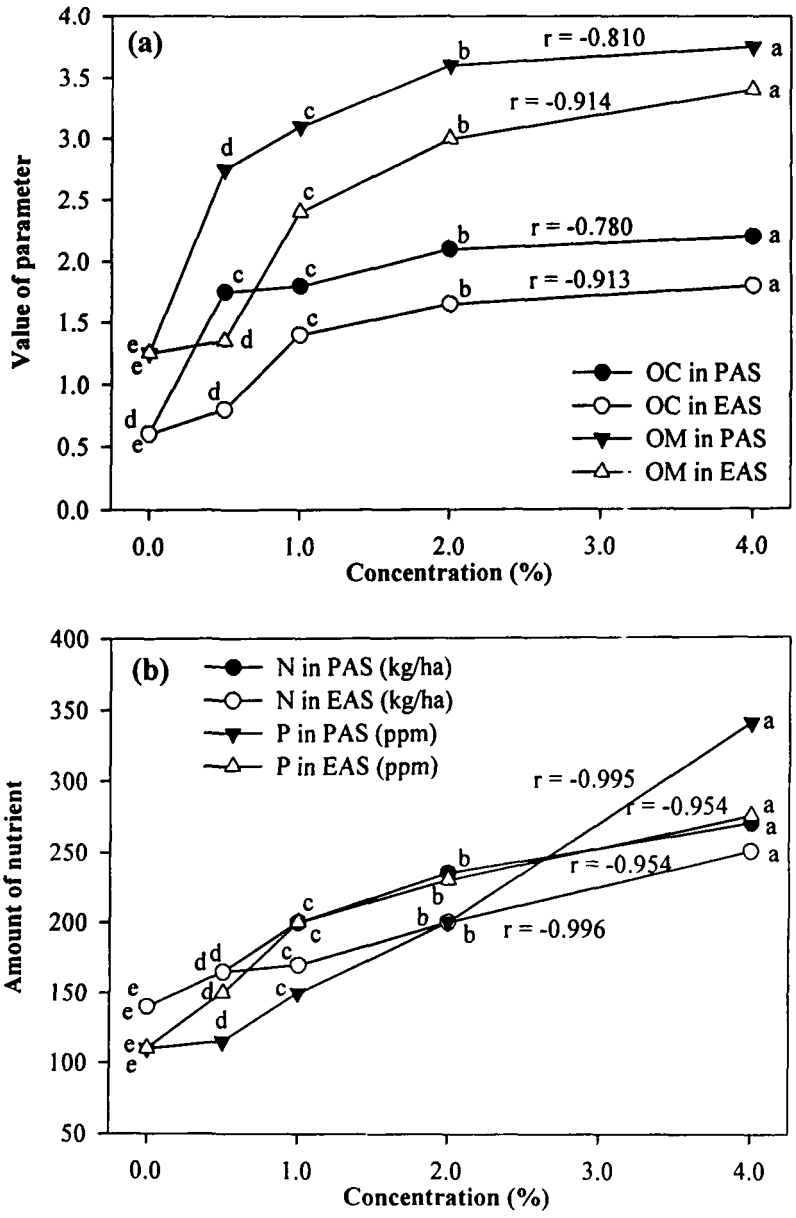
Property	US	PAS				EAS			
		0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0
pH	7.6^{a}	7.52^{a}	7.41^{b}	7.32^{c}	7.26^{d}	7.59^{a}	7.57^{a}	7.41^{b}	7.33^{c}
EC (mS)	0.49^{d}	0.56^{c}	0.60^{c}	0.96^{b}	1.57^{a}	0.55^{d}	0.82^{c}	1.08^{b}	1.43^{a}

EC: Electrical Conductivity; US : Unamended Soil (control); PAS: Powder Amended Soil; EAS: Extract Amended Soil. Different superscripts within a parameter in a row represent significant difference at $P<0.05$.

Not only the percent organic carbon content, even the amount of various macro and micronutrients were measured to be more in both powder and extract amended soil than respective control (Fig. 3.4a) Available N content in unamended soil was 142

kg/ha and it was more in both powder and extract amended soils. It increased by 1.94 and 1.86 times of control in 4% powder or extract amended soil (Fig. 3.4b).

Fig. 3.4. Effect of above-ground extracts or powder of *A. conyzoides* amended in soils on (a) organic carbon and matter content, and (b) amounts of nitrogen and phosphorus (PAS: Powder amnended soil; EAS: Extract amended soil).



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

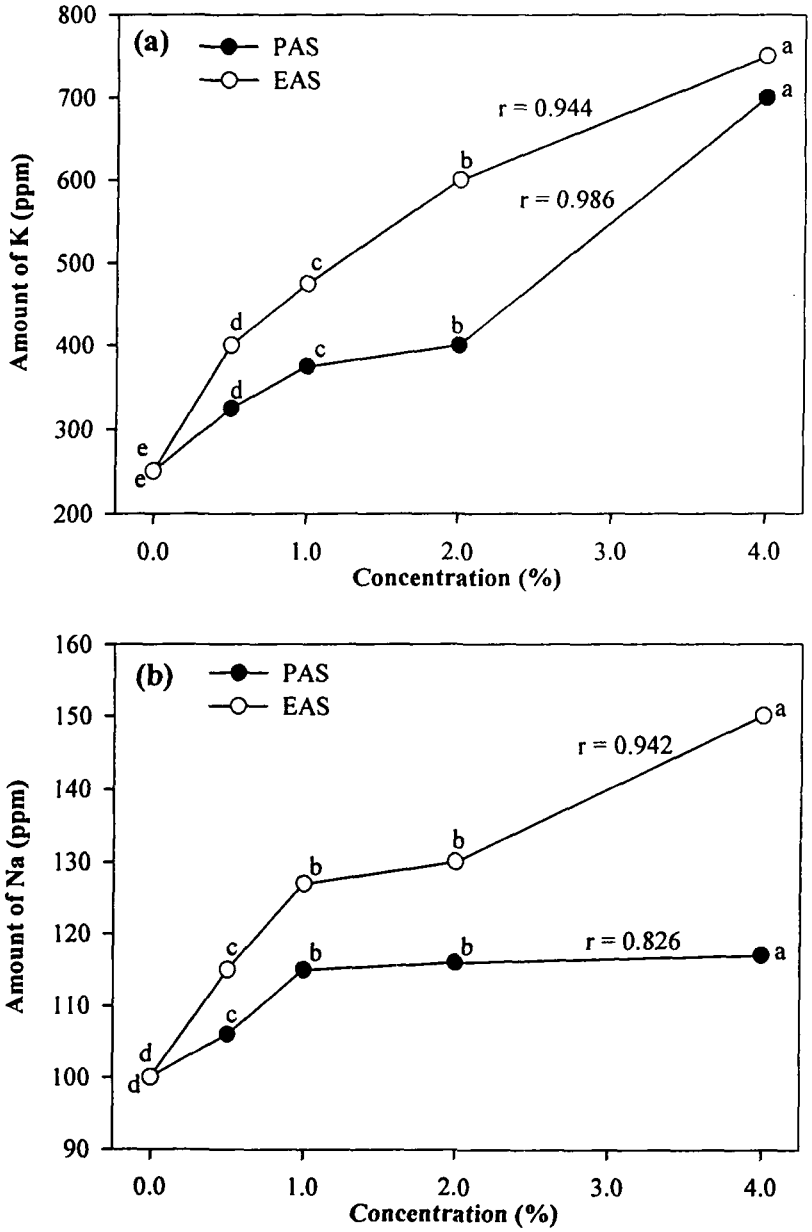
Almost similar trend was observed in available P content. In 4% powder amended

soil there was nearly 3 times increase in content of P over control. Almost similar observations were made in extract-amended soils though the increase in amount of available P was slightly lesser than in respective powder amended soil (Fig. 3.4b).

Likewise, available K and Na increased significantly in both powder and extract amended soils, respectively (Fig. 3.5a, b), though the extent of increase in Na was lesser compared to other nutrients. At 4 g powder concentration, K increased by nearly 3-times in both extract and residue amended soils whereas Na increased by around 1.2 times over that of control (Fig. 3.5a,b). Similar changes in amounts of Ca and Mg and Cl and HCO_3 were observed in the amended soils where the contents increased over that of unamended control soils (Fig. 3.6 a,b). In 4% extract or powder amended soils, amount of Mg increased 3 times of control (Fig. 3.6a). Amount of Cl increased by 3 times in soil amended with 4 g powder whereas it was 2.65 times more in extract amended soil. In general the increase was more in powder amended than in extract amended soils.

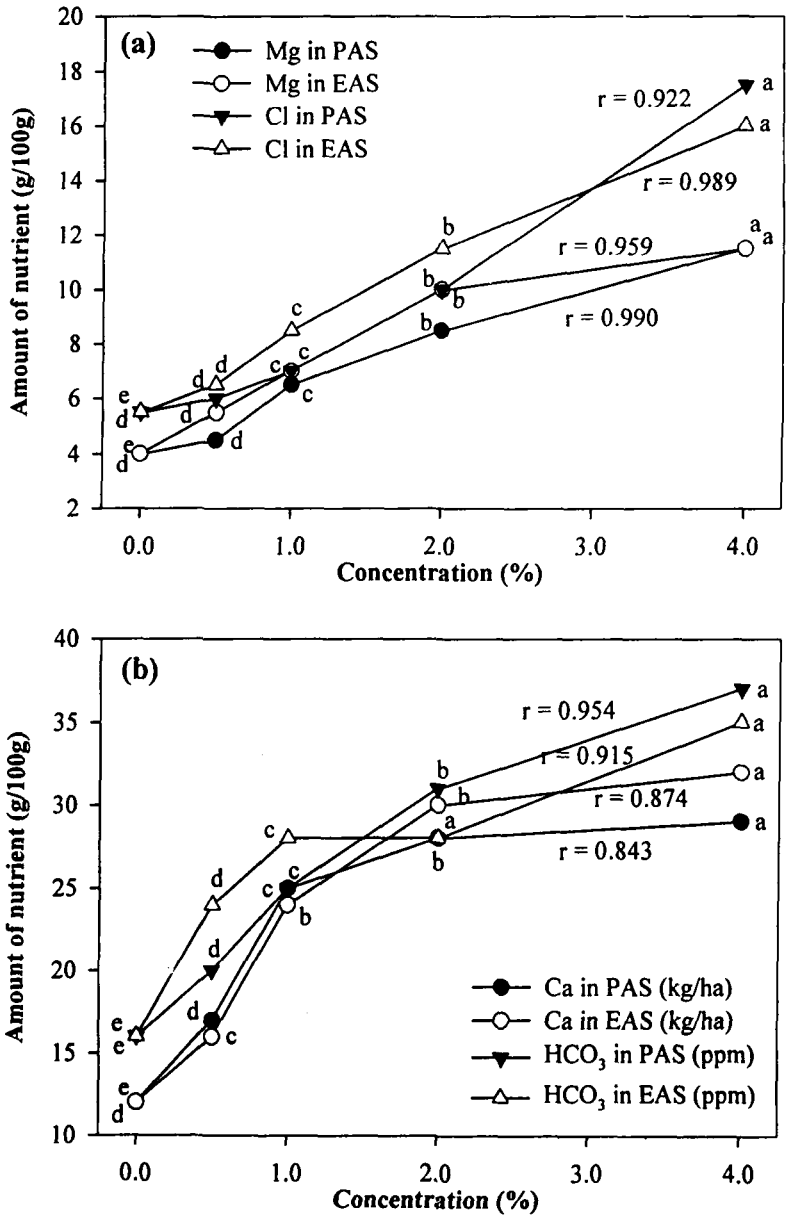
Not only the macronutrients even the amount of micronutrients (Cu, Zn, Mn and Fe) increased in the amended soils (Fig. 3.7a,b). In this case also, increase was more in powder amended soils than in extract amended soils at any concentration. Besides, chemical properties and amount of macro- and micronutrients, amount of total phenolics was also determined in amended soils. These also increased significantly with increasing amendment. Nearly, a five-fold increase in phenolics was observed when 4 g powder was amended, whereas in extract amended soils, increase was about 4 times (Fig. 3.8). Such a significant increase in phenolics could be detrimental to plant growth.

Fig. 3.5. Amount of (a) potassium and (b) sodium in soil soil amended with extracts or powder of above ground parts of *A. conyzoides* (PAS: Powder amened soil; EAS: Extract amended soils).



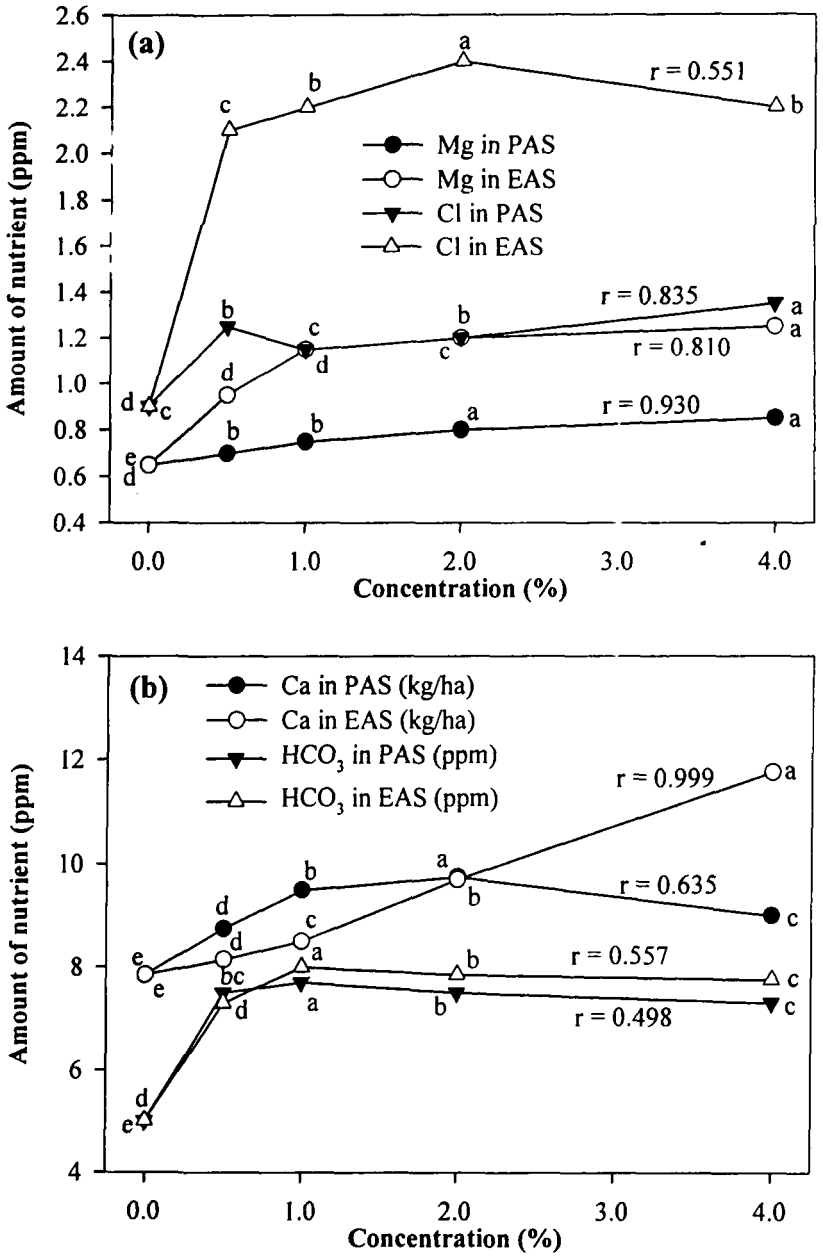
Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Fig. 3.6. Amounts of (a) magnesium and chloride, (b) calcium and bicarbonate ions in the soils amended with above-ground extracts or powder of *A. conyzoides* (PAS: Powder amnended soil; EAS: Extract amended soil).



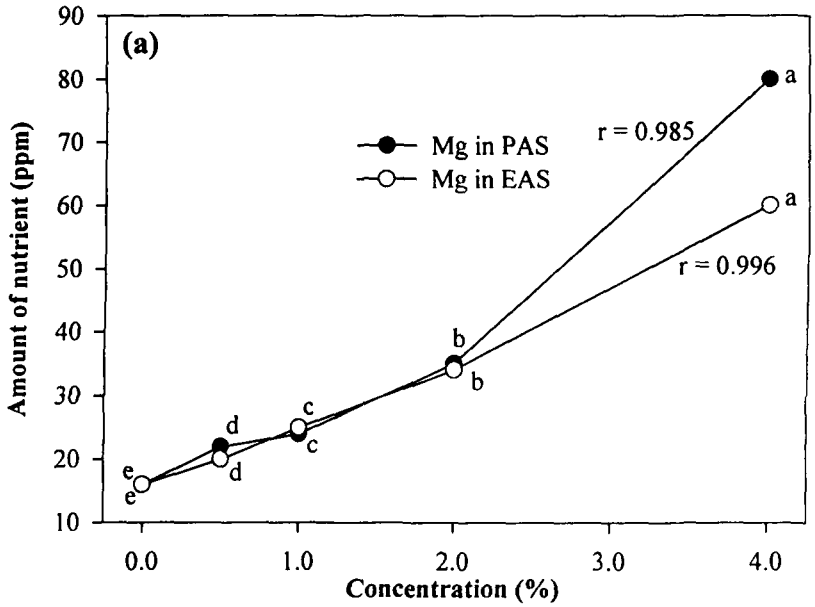
Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Fig. 3.7. Amounts of micronutrients in soils amended with extracts or powder of above-ground parts of *A. conyzoides* (PAS: Powder amnended soil; EAS: Extract amended soil).



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Fig. 3.8. Amounts of phenolics in soils amended with extracts or powder of above-ground parts of *A. conyzoides*.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Discussion

Ageratum conyzoides is an agricultural weed that has invaded various parts of India, including Bihar, Haryana, Himachal Pradesh, Punjab and Uttar Pradesh. Being agricultural weed, it can be commonly seen in croplands. The encroachment of the fields by the weed is especially more during the winter season that happens to be the growing period of the several crops. Consequently, the interference of the weed is the maximum with plants especially *Anagalis arvensis*, *Brassica oleracea* var. *botrytis* and *C. arietinum*. It is also known to interfere with plants *viz. P. mungo* and *O. sativa* etc. In the present study, above-ground parts of weed comprising main shoot and branches with flowers were collected when density of weed was high during its active growing period. These parts are generally cut and thrown into the field before or during the sowing of test plants. It was hypothesized that these cut portions dumped in the field cause phytotoxicity to the young growing test plants. Since leachation is the

most common and well-known mode of release of allelochemicals from the plant, the effect of aqueous extracts from above ground parts was studied towards test plants. Seedling length and dry weight were adversely affected especially at 2% and 4% extract concentration. The growth of *C. arietinum* was more affected compared to other test plants as none of the seeds germinated in 4% extract. It was followed by *O. sativa*, *Brassica oleracea* var. *botrytis* and *Anagalis arvensis.* Thus, extracts contained growth inhibitory metabolites, which were found to be phenolics a group of water-soluble allelochemicals. The pH and osmotic potential of extracts were, however, within the permissible range and hence did not play any role in retarding the test plants growth.

Since laboratory conditions may not be close to natural conditions because of disparity between concentrations selected for laboratory studies and actual natural concentrations (Harper, 1977), some growth studies were undertaken in the soil medium. For this purpose, soil medium was supplemented with extract of different concentrations ranging from 0.5% to 4%. The studies in soil medium amended with extract solutions indicated a drastic reduction in growth of the test plants, and weeds though the magnitude of reduction was not as much as with direct treatment of extract. Further, test plants were also grown in soil amended with powdered above-ground material of *A. conyzoides* in the varying concentrations. Here also reduction in growth of test plants was observed, thereby indicating that *A. conyzoides* imparts phytotoxicity to soil through its extract as well as its powdered material when directly added to the soil. In order to find out the reasons of growth reduction in soil in which extract or residues of above-ground parts were amended, various characteristics of soil and amount of nutrient were determined. The pH, conductivity, organic carbon

and organic matter were found to vary to the little extent. Hence the retardation of the growth does not seem to be on account of minor changes in the pH, conductivity or the organic carbon and organic matter. Likewise, no decrease in the amount of available macronutrients *viz.* N, P, *K,* Na, Ca and Mg was observed in amended soils. Rather, the amount of all tested nutrient increased significantly, but within the permissible range, thus unlikely to cause any toxicity. However, amended soils were found to have a significantly rich amount of phenolic acids compared to unamended control soil. These phenolics may act as putative allelochemicals. However, their amount was not as high as in case of extracts, which may be attributed to several reasons.

Blum *et al.* (1999); Kayoed (2006) have shown that phenolics enter soil medium and may undergo a number of changes *viz.* their adsorption to soil particles reversibly or irreversibly, leachation or translocation to lower soil horizons, microbial degradation thus leading to changes in structure and chemical nature and consequently alteration of phytotoxicity. Environmental conditions further play an important role in the availability of phenolics in the soil or in other words, their phytotoxic effect on other plant (Einhellig, 1996; Hong *et al.,* 2006; Koloren, 2006; Dandelot *et al.*, 2008).

Available amount of phenolics in the soil was significantly lesser than in plant extracts prepared under laboratory conditions. The observed phytotoxicity in soil medium with extract or powder of above-ground parts of the weed, thus, is a function of available phenolics in the soil. Under natural conditions, because of availability of plant, a continuous flux of phenolics is maintained in the soil that leads to plant injury. Since any of the nutrients studied was neither found to be limiting nor in the

toxic levels, the presence of phenolics could, therefore, be the sole reason of growth reduction in the test plants. It is, thus, concluded that above-ground parts of *A. conyzoides* impart phytotoxicity to the soil by releasing phenolics that in turn result in the growth reduction of some test plants.

EXPERIMENT – 4

Objective

To study the comparative phytotoxicity of green and brown leaves (usually present on the lower part of the stem) of *Ageratum conyzoides.*

Hypothesis to be Tested

Two types of leaves i.e. fresh green and partially decayed brown leaves are present on mature plants of *A. conyzoides.* It is assumed that both types of leaves contribute towards the phytotoxic potential of *A. conyzoides.* So, the present study was carried out to evaluate their comparative phytotoxicity towards some test plants.

Parameters Studied

Seedling growth of test plants (*Anagalis arvansis*, *Brassica oleracea* var. *botrytis*, *C. arietinum*, *Phaseolus mungo and Oryza sativa*) in terms of their respective lengths and dry weights was evaluated in response to extracts and soils amended with powder of each type of leaf. Further, changes in soil characteristics in terms of pH, conductivity, and various macro and micronutrients (in amended and unamended control soil) were also studied.

Methodology

Fresh green and decaying brown leaves were collected from the plants of *A. conyzoides.* These were dried separately, powdered and labeled as green leaf powder (or GLP) and brown leaf powder (or BLP). From these powders, 2% aqueous extracts were prepared and referred as green leaf extracts (or GLE) and brown leaf extracts (or BLE), respectively. These were further diluted so as to get 1% solution of each. These aqueous extracts of 1 or 2% concentrations were used to study the early growth of test

plants under laboratory conditions.

Further, each type of leaf powder was mixed into soil at the rate of 1 and 2 g/100g soil and labeled as green leaf soil (or GLS) and brown leaf soil (or BLS). Amended or control soil (where no amendment was made) were filled in the pots in which seeds of test plants were sown and the effect on seedling length and dry weight was studied after 8 days. For each treatment, five replicates were maintained. Further, pH, conductivity and amount of total phenolics were determined in extracts as well as in amended soils. Likewise, changes in amount of macro- and micronutrients were studied in amended soils as per methods described in chapter Material and Methods.

Results

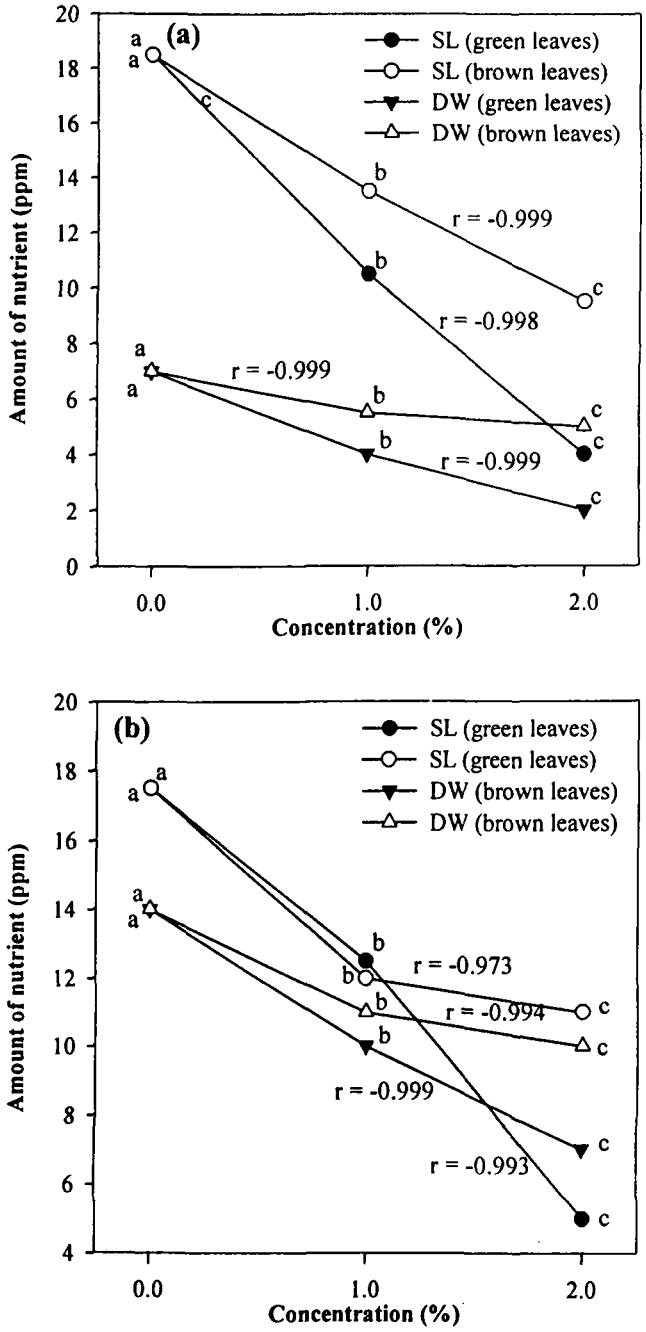
a) Effect of Extracts

The seedling length of *Anagalis arvensis* in control was measured to be 18.22±0.27 cm. It decreased significantly in response to 1 or 2% GLE or BLE, however, the decrease from control was more in GLE. At 2% GLE, the seedling length was reduced by 78% compared to 50% in BLE. In other words, the length of seedlings treated with GLE was 60% lesser than that in BLE (Fig. 4. 1 a).

Likewise, dry weight of *Anagalis arvensis* seedlings was also found to be less in response to both GLE and BLE compared to that in water treated control. Dry weight of seedlings in control was measured to be 6.25±0.3 mg. Here also, reduction was more in GLE treated seedlings (Fig. 4. 1a). The difference in the growth of seedlings treated with GLE or BLE both in seedling length and dry weight was statistically significant when analyzed by *t*-test. The values of correlation coefficient between concentration and seedling length, or dry weight were highly significant and reciprocal. The seedling length and dry weight *Brassica oleracea* var. *botrytis* were

significantly reduced when treated with different concentrations of GLE or BLE (Fig. 4.1b), Here also the value of *r* between concentration of GLE or BLE and parameter was found to be statistically significant.

Fig. 4.1. Effec of aqueous extracts of green and brown leaves of *A. conyzoides* on seedling length (SL) and dry weight (DW) of (a) *A. arvensis* and (b) *B. oleraceae* var. *botrytis.*



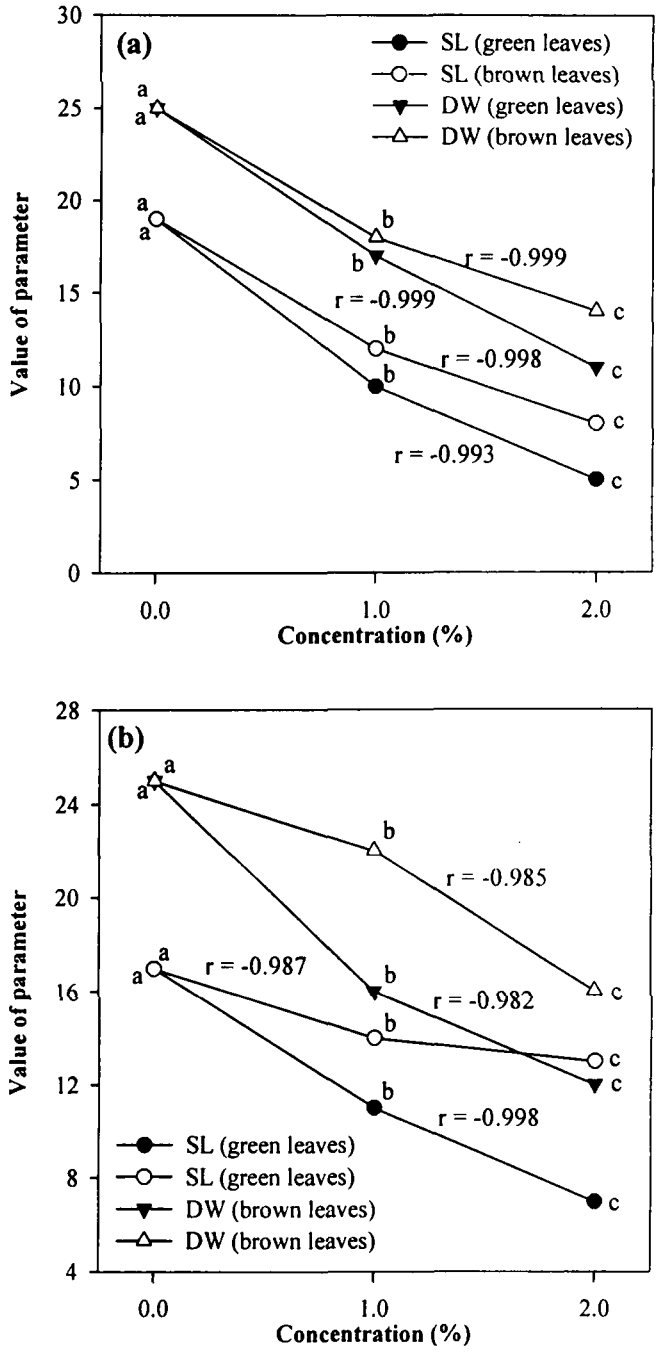
Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

In *C. arietinum*, almost similar trend was observed with treatments of both GLE and BLE. At 1% concentration, difference in values of seedling length or dry weight was statistically insignificant when treated with GLE or BLE (Fig. 4.2a). However, at 2%, it was found to be significant with respect to both the parameters (Fig. 4.2a). In *P. mungo*, seedling length and dry weight were measured to be less compared to control when treated with 1 or 2% GLE or BLE. In dry weight, effect of GLE was more than BLE and this difference was significant statistically at 1% concentration (Fig. 4.2b). Almost similar observations were made with respect to seedling length. Likewise, in *O. sativa,* irrespective of concentration, inhibition in seedling length was more (statistically significant) in response to treatment of GLE, compared to BLE. In case of dry weight, the observations were similar to those of seedling length at 1% concentration of GLE or BLE. At 2% concentration, however, no difference in dry weight was observed when treated with either GLE or BLE. However, it was lesser than at 1% concentration or at control (Fig. 4.3).

b) Growth in Amended Soils

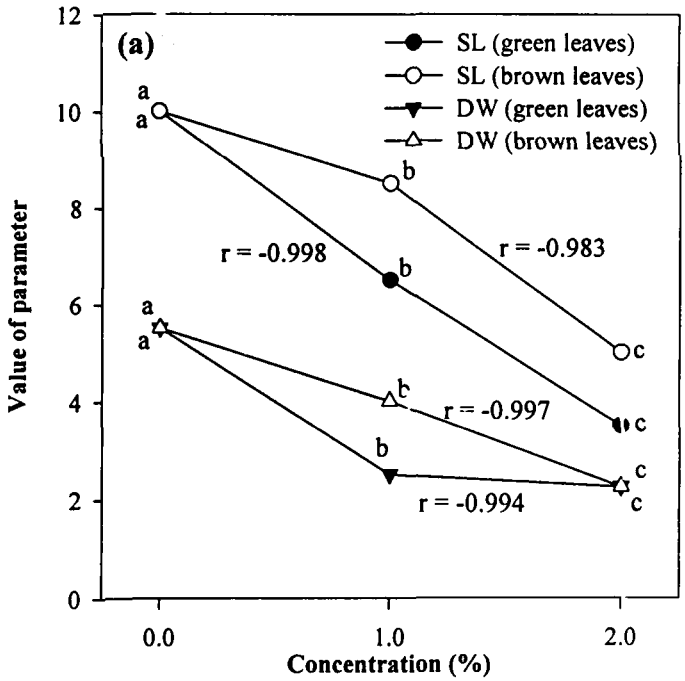
The growth of test plants was also studied when soils were amended with green or brown leaves of *A. conyzoides.* In *Anagalis arvensis* both seedling length and dry weight were less than control in samples treated with in 1% BLS or GLS. Among the two treatments, the decrease was more in GLS than BLS. In case of those treated with 2% of either of the GLS or BLS, the seedling length further decreased. At this concentration, the similar observation was also made with respect to dry weight (Fig. 4.4a). Likewise, in *Brassica oleracea* var. *botrytis* both seedling length and dry weight were measured to be short/less, when grown in GLS or BLS. The reduction

Fig. 4.2. Effect of aqueous extracts of green and brown leaves of *A. conyzoides* on seedling length (SL) and dry weight (DW) of (a) *C. arietinum* and (b) *P. mungo.*



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Fig. 4.3. Effect of aqueous extracts of green or brown leaves of *A. conyzoides* on seedling growth of *O. sativa.*

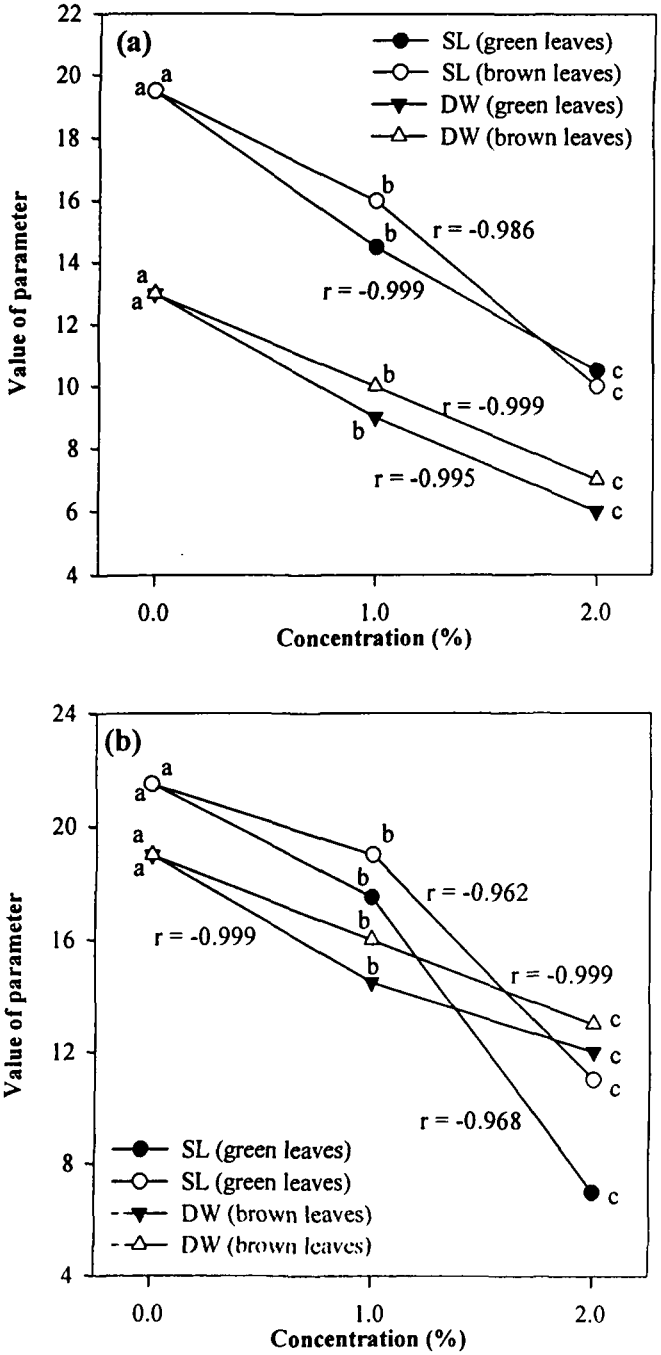


Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

was relatively more at 2% concentration (Fig. 4.4b). The difference in growth with, respect to both seedling length and dry weight was statistically significant and correlation coefficient values between concentration and parameters in GLS and BLS were statistically significant.

Almost similar trend of changes were observed in *C. arietinum* and *P. mungo*. Here, the reduction was more in GLS compared to BLS and it was statistically significant at 2% extracts (Fig. 4.5a, b). Further, dry weight of both the test plants was less when grown in GLS and BLS. However, reduction was relatively more in GLS than BLS and the difference between the two was statistically significant only at 2% concentration (Fig. 4.5 a, b). In each case, the value of correlation coefficient was negative but strong.

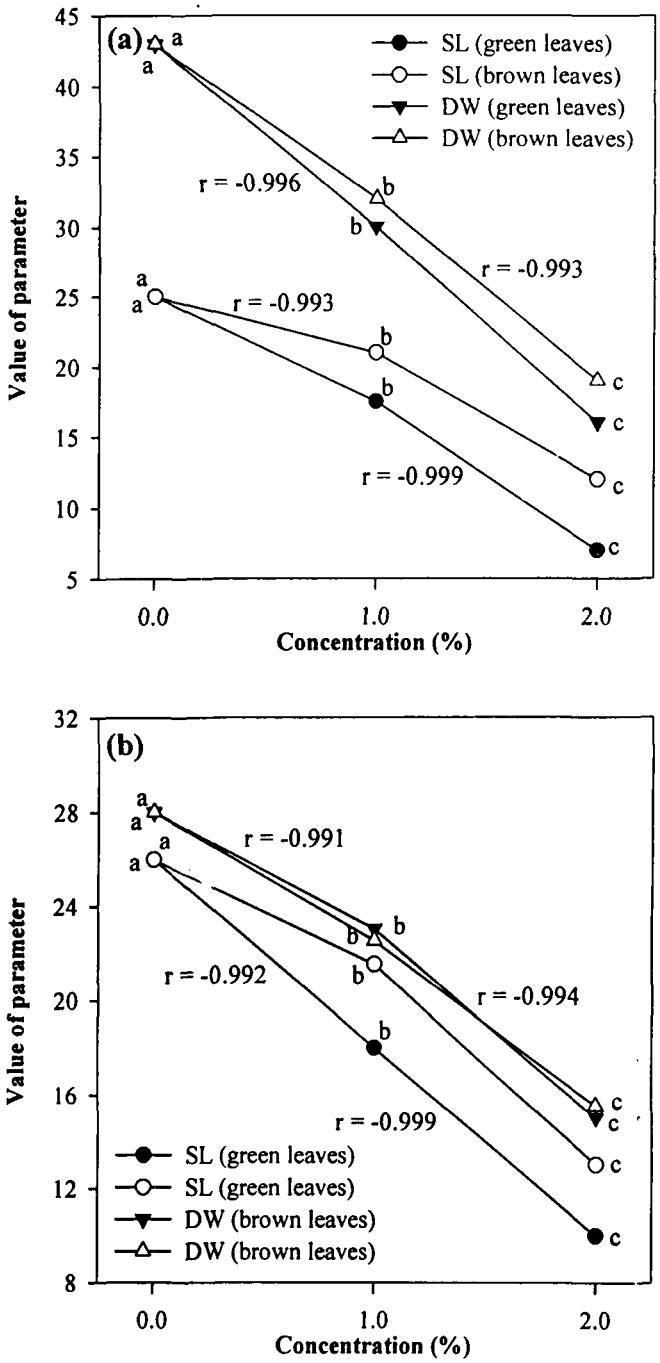
Fig. 4.4. Effect of green or brown leaf powder of *A. conyzoides* (amended in soil) on seedling length (SL) and dry weight (DW) of (a) *A. arvensis* and (b) *B. oleraceae* var. *botrytis*.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Likewise, in *O. sativa*, both seedling length and dry weight, compared to untreated control, were reduced when grown in GLS or BLS (Fig. 4.6).

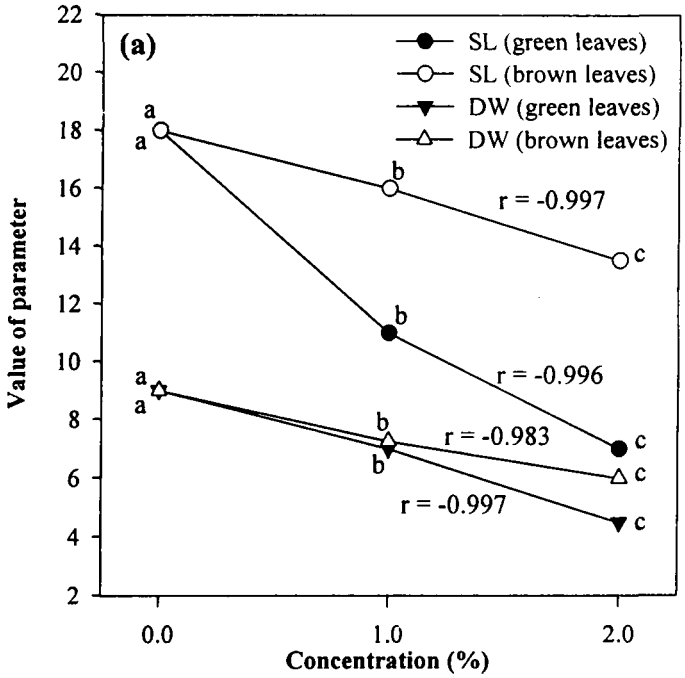
Fig. 4.5. Effect of green or brown leaf powder of *A. conyzoides* (amended in soil) on seedling length (SL) and dry weight (DW) of (a) *C. arietinum* and (b) *P. mungo*.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Appreciably more decrease in seedling length was noticed in GLS and this decrease was statistically significant (than in BLS) both at 1 and 2% concentrations.

Fig. 4.6. Effect of green or brown leaf powder of *A. conyzoides* on (amended in soil) on seedling length (SL) and dry weigh (DW) of *O. sativa.*



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

As regards dry weight, however, not much difference between GLS and BLS treatment was observed. Differences what so ever between the two treatments were statistically insignificant at 1% though compared to control decrease in both was statistically significant. The values of *r* were highly significant and exhibited reciprocal relationship (Fig. 4.6).

c) Changes in Chemical Properties

Based on the laboratory study involving GLE and BLE, where a strong inhibitory effect was observed, both extracts were analyzed for pH, conductivity and total phenolic content. In GLE or BLE, pH was nearly neutral at 1% concentration, whereas it was slightly acidic at 2% concentration. However, these differences were statistically significant between GLE and BLE (Table 4.1). Conductivity, however,

sharply increased both in GLE and BLE with increase in concentration, though it was lesser in BLE (Table 4.1). Osmotic potential of both GLE and BLE was almost the same: at 1% concentration. However at 2% concentration, it was significantly lesser in BLE compared to GLE. Both BLE and GLE were found to contain an appreciable amount of phenolics, though the amount was significantly lesser in BLE. At 2% concentration, the amount In BLE was less than half of that in GLE (Table 4.1).

Table 4.1 Values of pH, conductivity, osmotic potential and phenolic content in green leaf (GLE) and brown leaf (BLE) extracts of *A. conyzoides*.

Parameter	GLE (%)		BLE (%)	
	1	2	1	2
pH	6.56^{a}	6.26^{c}	6.59^{a}	6.37^{b}
Conductivity (mS)	1.20^{c}	2.66^{a}	1.13^{d}	1.93^{b}
Osmotic potential (-bars)	0.43^{c}	0.96^{a}	0.41^{c}	0.69^{b}
Amount of Total phenolic (µg/ml)	346.36^{b}	760.18^{a}	156.90^{d}	306.11^{c}

Different superscripts within a row represent significant difference at $P<0.05$.

Table 4.2. Changes in pH, conductivity and osmotic potential in soils amended with green (GLS) or brown leaves (BLS).

Parameter	Unamended soil	GLP (g/100g soil)		BLP (g/100g soil)	
		1	2	1	2
pH	7.59^{a}	7.35^{b}	7.18^{d}	7.38^{b}	7.22^{c}
Conductivity (mS)	0.53^{c}	1.42^{b}	1.81^{a}	0.70^{d}	0.78^{c}
Osmotic Potential (-bars)	0.19^{d}	0.51^{b}	0.65^{a}	0.25^{c}	0.28^{c}

Different superscripts within a row represent significant difference at $P<0.05$.

In comparison to the extracts where the values of pH were less than 7.0, the soils amended with GLP or BLP have pH more than 7.0. The unamended control soil exhibited the value of 7.59 - significantly more than the amended soils. Since an

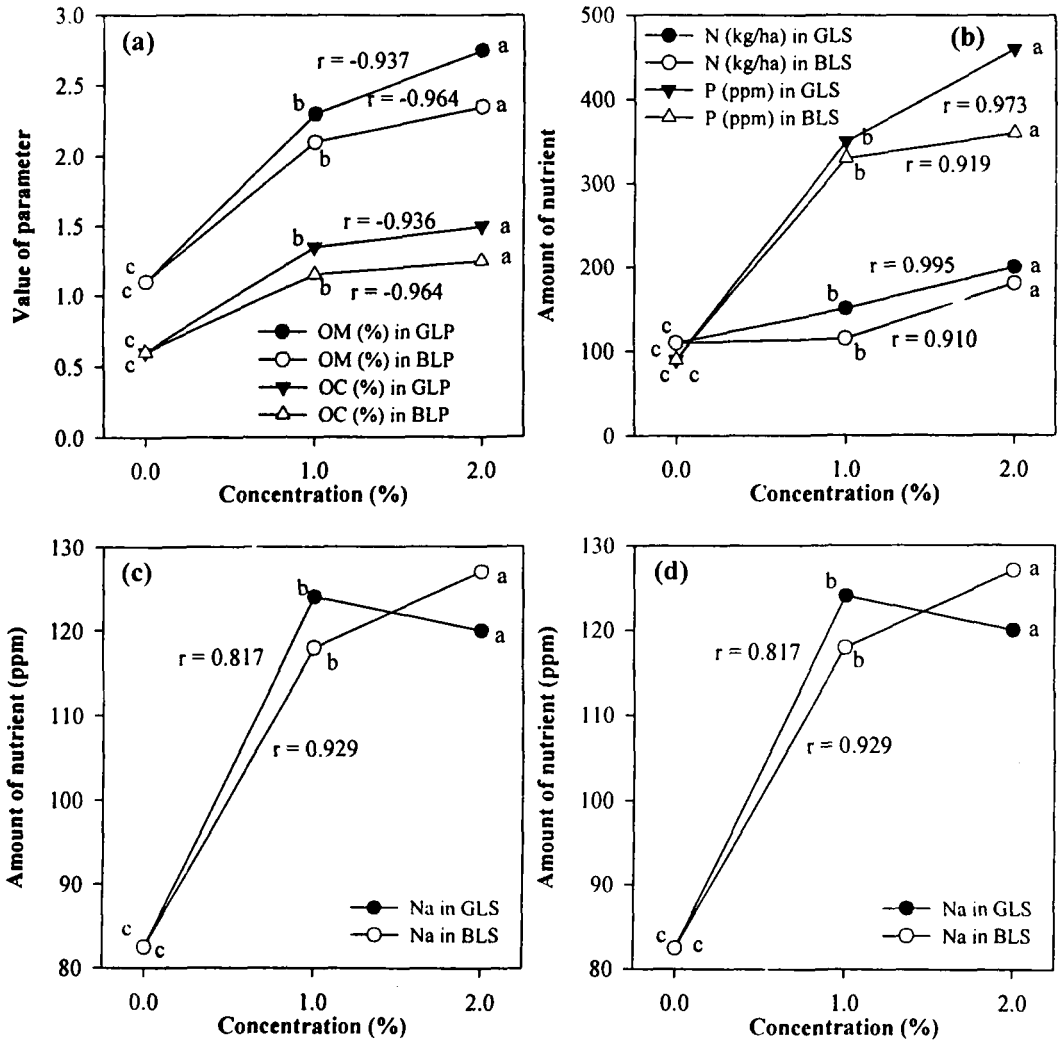
appreciable reduction in growth of test plants was observed in amended soils, it was thought to analyze these soils for presence of inhibitory principles and available nutrients. In amended soils, pH was measured to be less, compared to unamended soil and the value further decreased with increasing concentrations (Table 4.2). At respective concentrations, the differences between GLS and BLS were statistically insignificant. Between the two concentrations, the pH was relatively low (towards acidic) in soils amended with, 2% of green or brown leaf powder. However, conductivity of unamended soil was very low (0.53 mS). After amendment it increased more in GLP than in BLP amendments. In BLP amendments, it was less than half of that in GLP and increased with increasing rate of amendment. The difference between conductivity values of GLS and BLS was statistically significant (Table 4.2). Almost similar trend of changes were observed with osmotic potential.

The amounts of organic carbon and organic matter in the unamended control soil were measured to be 0.62% and 1.07%, respectively. Upon addition of 1g of GLP or BLP, amount of both OC and OM was seen to increase and increased further with amendment (Fig. 4.7a). The values of OC and OM were insignificantly less in BLS compared to GLS as respective concentration. However, the differences between the values of GLS and BLS were significant. Correlation coefficient values were calculated to be positive and strong in both the cases.

Likewise, the values of N, P (Fig. 4.7b) and K, Na (Fig. 4.7c,d) were significantly more in the amended soils and their amount increased significantly with increasing concentration. However, difference in amount of Na between GLS and BLS was insignificant. Similar observations were made in case of Ca, Mg (Fig. 4.8a). The increase in amount of Ca was significantly more in GLS than in BLS whereas in

Mg, increase was almost same in each. Likewise, the amount of Cl was significantly lesser in BLS compared to GLS. In case of HCO_3, almost similar values were measured in GLS and BLS at 1% concentration whereas at 2% concentration, the amount was significantly lesser in BLS than in GLS (Fig. 4.8b). Likewise, amount of micronutrients (Cu, Zn, Mn and Fe) was more in amended soils than the unamended

Fig. 4.7. Changes in (a) organic carbon, organic matter, and available (b) nitrogen, phosphorus (c) potassium (d) sodium after amendment of green or brown leaves in soil.

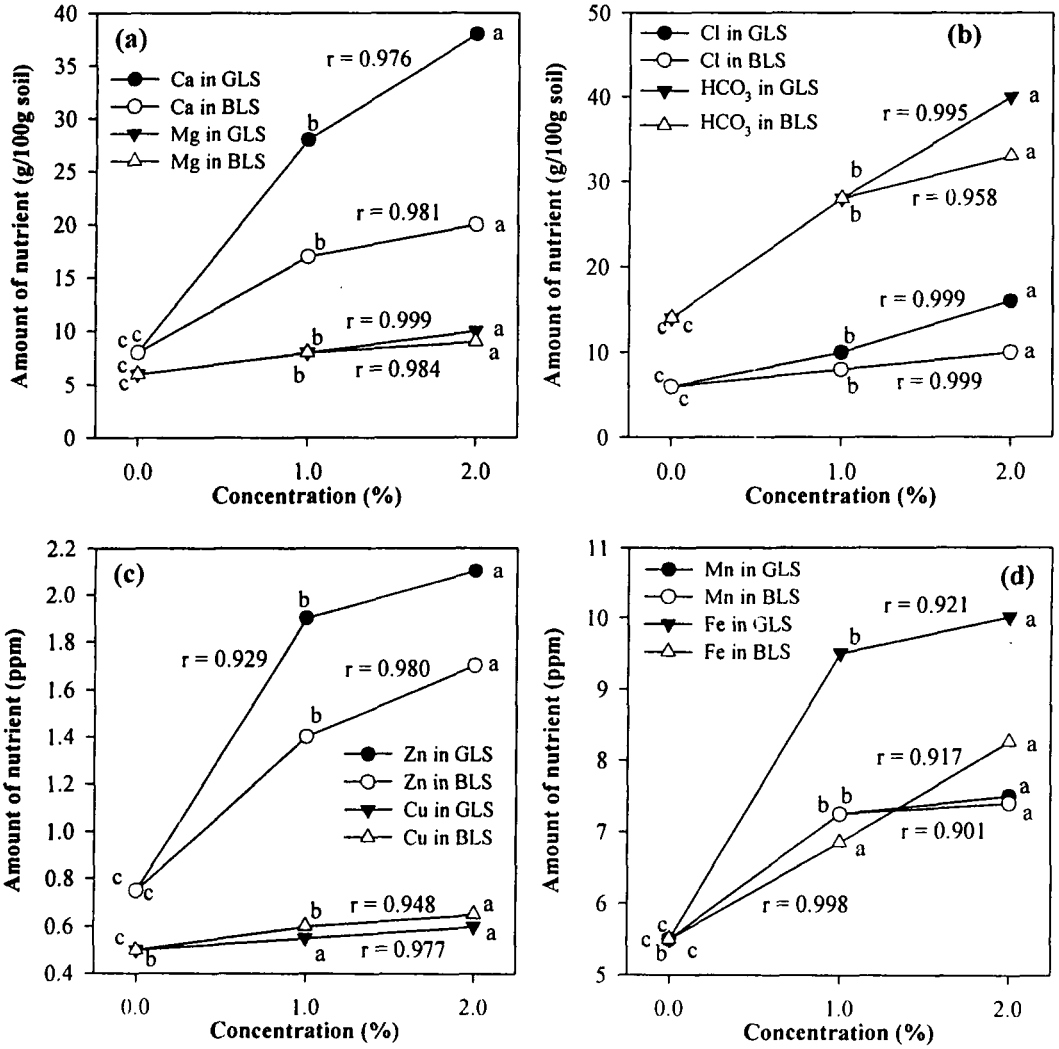


Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

control soil (Fig. 4.8 c, d). There was not much change in amount of Cu between control and BLS or GLS whereas the amount of Zn increased significantly even at

lower concentration of each BLS or GLS. The amount of Zn was significantly more in GLS compared to BLS even at lower concentrations of amendment (Fig, 4.8c), Almost similar trend of changes was observed in Fe (Fig. 4.8d). However, in Mn, the difference between values in GLS and BLS was insignificant.

Fig. 4.8. Changes in different macro and micronutrients after amendment of green and brown leaves in soil.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

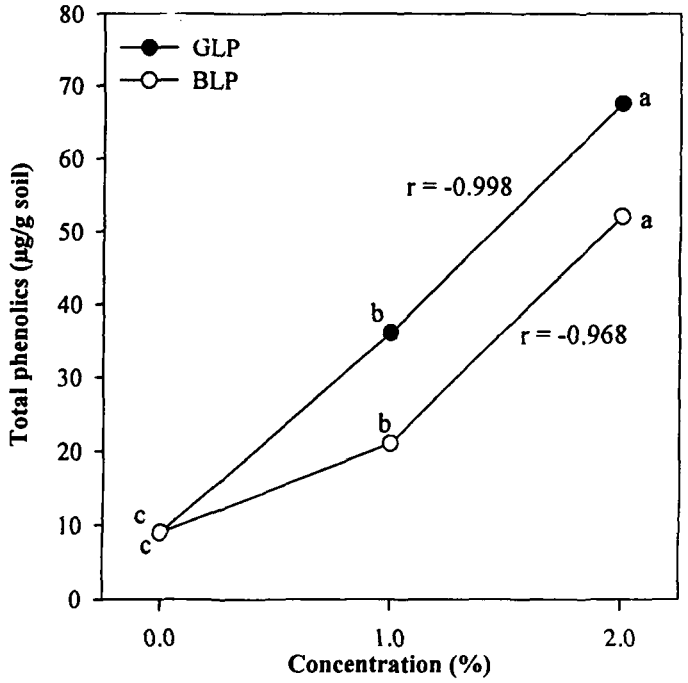
Further, the amount of water-soluble phenolics increased significantly in amended soils compared to unamended soil. The amount of phenolics increased with increasing concentration in both GLS or BLS and it was significantly lesser in BLS

compared to GLS (Fig. 4.9).

Discussion

Both green and brown leaves of *A. conyzoides* were found to be phytotoxic in nature when growth studies were conducted on filter paper in Petri dish or in soils

Fig. 4.9. Changes in phenolic content after amending green or brown leaf powders in soil.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

amended with them. Based on those studies, it was speculated that leaves may contain water-soluble phytotoxins allelochemicals that interfere with the growth of test plants. Lesser phytotoxicity of brown leaves could be attributed to the loss of allelochemicals during the decaying process. In order to explore the reasons and possible nature of allelochemicals, several tests were conducted in extracts as well as in amended soils. In case of extracts, changes in pH and conductivity were not the possible reasons as change in them was such that it is unlikely to cause any effect on growth. Thus, the possible role of some water-soluble allelochemicals was explored. Since phenolics are

the most commonly found water-soluble allelochemicals (these were found in earlier experiments also), these were quantified in the extracts. Their amount was substantially high, in both GLE and BLE. Among them, these were significantly more in GLE compared to BLE. Therefore, presence of phenolics could be the possible reason for observed growth reduction in the test plants.

As already speculated, amount of phenolics was significantly less in BLE compared to GLE that also correlated with reduction in growth. In the soils amended with GLP and BLP, similar growth reductions in test plants were observed. Here also, the presence of phenolics, which were incidentally found to be quite high in amended soils, could be one of the possible reasons. Further, the amount of macro- and micronutrients which were determined in amended and unamended soils indicated that amount of all the nutrients were more in amended soils and they increased with the increase in rate of amendment. Since phenolics are known to interfere with macro and micronutrients (Appel, 1993); here also these could affect the uptake of nutrients by the growing seedlings (Glass, 1973; Bergmark *et al.*, 1992; Lyu and Blum, 1990; Samedani and Baghestani 2005; Punjani *et al.*, 2006; Srisa-Ard, 2007). Thus, phenolics may be responsible directly for observed growth reduction or indirectly by affecting uptake of nutrients in the amended soils.

EXPERIMENT – 5

Objective

To study the influence of soil texture on the phytotoxicity of *Ageratum conyzoides*.

Hypothesis to be Tested

Soil texture may play an important role in determining the fate of allelochemicals as allelochemicals upon release may either adsorb to soil particles or may undergo transformation depending upon environmental conditions. Thus, allelopathic nature of plants varies under different soil conditions.

Parameters Studied

Growth studies were conducted by taking *Oryza sativa* as a test plant. The parameters under study included length and dry weight of eight days old seedlings. Besides this, these amended soils were also analyzed for pH, conductivity, amount of total phenolics and some available macronutrients.

Methodology

On the basis of soil particle size, five types of soils i.e. sandy (S), clayey (C), loam (L), sandy loam (SL) and clayey loam (CL) soils were used to study the effect of soil texture on the phytotoxicity of *A. conyzoides*.

To these five types of soils, green leaf powder of *A. conyzoides* was incorporated in the ratio 1 or 2 % (w/w). Likewise, for each type of soil, one set was kept as control where no amendment was done. Each type of soil, whether unamended or amended, was filled in plastic pots (as explained in material and methods). For each treatment five replicates were maintained. Healthy and fresh seeds of *O. sativa* were

sown in these pots. After eight days, growth was measured in terms of seedling length and seedling dry weight.

These soils (amended or unamended) were also analyzed for some physical and chemical properties of soil. These included pH and electrical conductivity (EC), organic carbon (OC), organic matter (OM) and available macronutrients and amount of total phenolics as per Swain and Hillis (1959). The whole experiment was repeated and mean of data was analyzed by one-way and two-way ANOVA.

Results

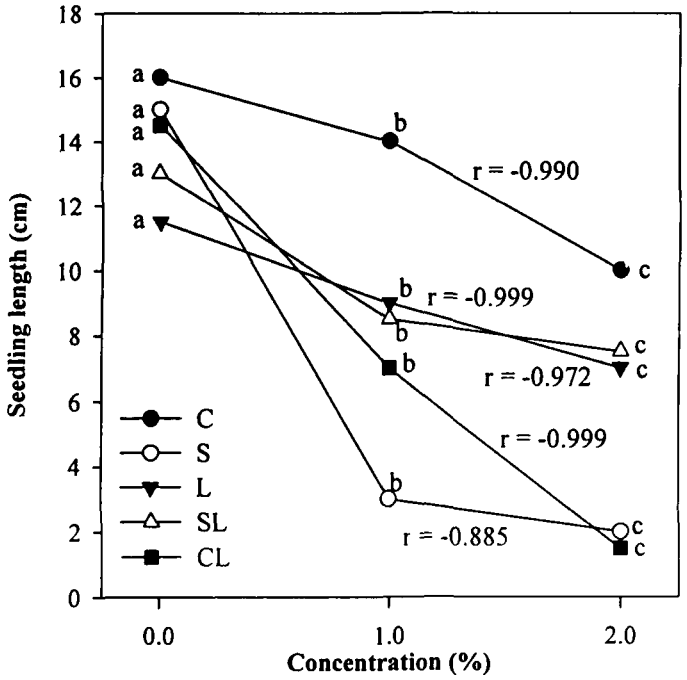
a) Seedling Length

A variable response of *O. sativa* sown in unamended different textured soil was observed. Maximum seedling length (16.05 cm) was measured when sown in clayey soil followed by sandy soil (15 cm). In sandy loam and clayey loam soil, the seedling length was 13.0 cm and 14.5 cm, respectively, whereas in loam alone it was 11.46 cm.

In each type of soil (i.e. sandy, clayey, loam, sandy loam and clayey loam) amended with different concentrations of *A. conyzoides* leaf powder, the seedling length of *O. sativa* was measured to be shorter than respective controls. With increasing rate of amendment, a further reduction in seedling length of *O. sativa* was observed (Fig. 5.1). In clayey soil, the seedling length was reduced by nearly 17 and 54%, respectively, when amended with 1 or 2% leaf powder. In sandy soil, however, the reduction was too high and more than 80% in both 1 and 2% leaf powder amended soils (Fig. 5.1). In loam soil amended with 1 or 2 g leaf powder, the seedling length was 8.85 and 5.83 cm, respectively, compared to control (11.46 cm), thus exhibiting a reduction of nearly 23 and 41%, respectively. In sandy loam soil, the seedling length in soil amended with 1% leaf powder was measured to be 8.18 cm (50% reduction

compared to control). Further, in the soil amended with 2% leaf powder, the reduction in seedling length was maximum i.e. nearly 92% (Fig. 5.1). In clayey loam, the seeding length was reduced by 37% and 53% at 1 or 2%, respectively.

Fig. 5.1. Seedling length of *O. sativa* in soils of different textures amended with leaf powder of *A. conyzoides*.

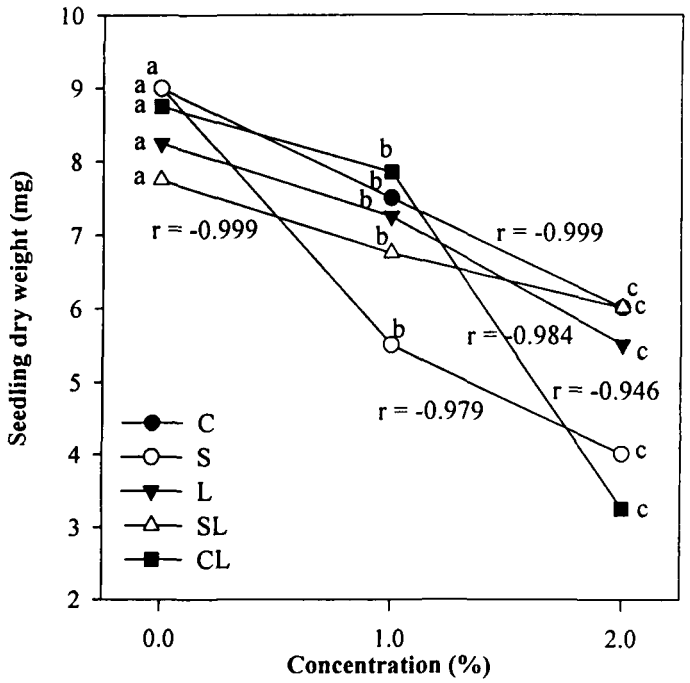


Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Like seedling length, the dry weight of *O. sativa* seedlings sown in different types of unamended soils, also, exhibited variation although not as high as seedling length. Here, the dry weight was measured to be maximum in clayey as well as sandy soil with a minor difference (8.92 and 8.91 mg, respectively; Fig. 5.2). The dry weight in clayey loam and loam was measured to be 8.64 and 8.17 mg, respectively. Least dry weight of seedlings was measured in sandy loam (7.74 mg). Compared to these values, dry weight of *O. sativa* seedlings decreased in amended soils and more inhibition was observed with higher concentration of leaf powder amendment i.e. 2%. As in case of

seedling length, here also, maximum inhibition was observed in sandy loam (nearly 63%) followed by sandy soils (nearly 59%) when amended with 2% leaf powder. In case of clayey soil and loam amended with 2% leaf powder, reduction in dry weight of seedlings was found to be approximately same i.e. nearly 37 and 36 % respectively. In case of clayey loam, the dry weight was reduced only up to 27% and 14%, at 2% or 1% concentration of leaf powder, respectively (Fig. 5.2). Thus, a considerable reduction in dry weight was observed when sown in different types of soils on the basis of texture.

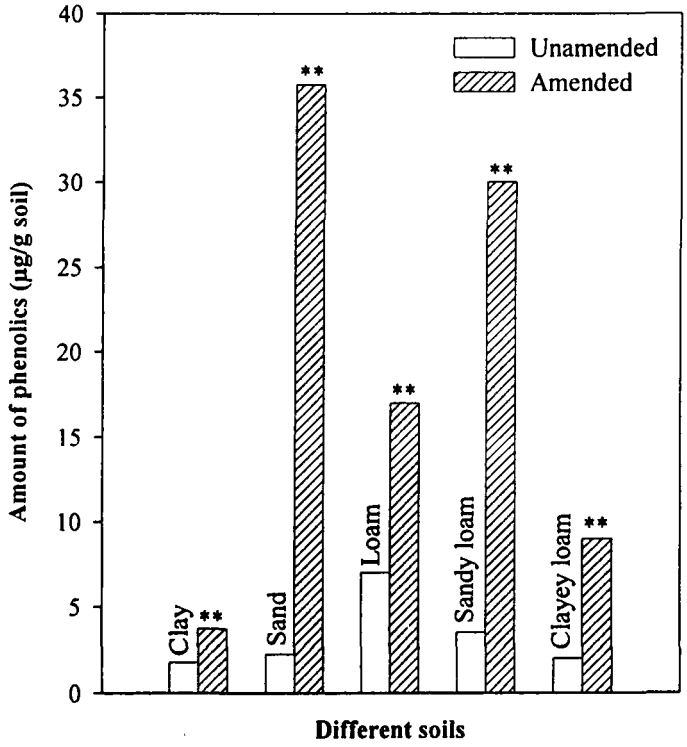
Fig. 5.2. Seedling dry weight of *O. sativa* in soils of different textures amended with leaf powder of *A. conyzoides*.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Since, both seedling length and seedling dry weight were affected by soil texture as well as with different concentrations of leaf powder, the data were put to 2-way analysis. Interaction between concentration and soil texture, in terms of seedling

Fig. 5.3. Changes in amount of total phenolics in soils of different texture upon amendment of leaf powder of *A. conyzoides*.



length and seedling dry weight was measured to be significant indicating, thereby, that both the attributes affected seedling length and dry weight significantly at $P < 0.05$ (Table 5.2).

Table 5.1 Composition of different textured soils used for present experiment.

Coarse Sand (2-0.2 mm) (%)	Fine sand (0.2 – 0.02 mm) (%)	Slit (0.02 – 0.002 mm) (%)	Clay (<0.002 mm) (%)	Textured defined
10.04±1.23	14.34±1.69	34.79±4.89	40.83±5.80	Clay (C)
36.43±4.34	42.71±6.02	8.62±0.98	12.24±1.21	Sand (S)
26.20±1.81	24.32±1.79	27.21±2.05	22.27±1.81	Loam (L)
13.34±1.43	21.68±1.74	36.35±5.05	28.63±4.46	Clayey Loam (CL)
26.74±2.86	38.83±5.43	24.13±5.43	10.30±1.03	Sandy Loam (SL)

Table 5.2 Interaction between concentration of leaf powder and soil texture and their effect on phototoxicity of *A. conyzoides*.

Parameter	F-value (soil texture)	F-value (concentration)	F-value (soil texture × concentration)
Seedling length	308.22	2096.42	111.93
Seedling dry weight	16.57	441.22	15.59

Further, these amended soils were analyzed for different nutrients and some other properties *viz.* pH, conductivity, organic carbon (OC) and organic matter (OM) and amount of phenolics. There was a significant difference in soil pH, conductivity, OC and OM with respect to different types of soils. The pH of both sandy and sandy loam soils was significantly more than other soil types and was the minimum in clayey soil. However, soils amended with 2% leaf powder had significantly lesser pH compared to respective controls. Conductivity of unamended soils also differed appreciably. It was the maximum in clayey loam and minimum in sandy soil. On the other hand, conductivity increased significantly in amended soils and the maximum values were observed in sandy loam, clayey loam and loam where it was more than 1000 μS. In contrast, sandy and clayey soil, values of conductivity were not too high but increased 4 to 5.5 times in amended soils compared to their respective controls (Table 5.3). Likewise, organic carbon and organic matter were also measured to be more when soils were amended with leaf powder of *A. conyzoides*. However, great variation in organic carbon and organic matter was observed in different types of soils whether amended or unamended. Increase in organic carbon was the maximum in sandy and sandy loam soil (5.0 and 4.3 times, respectively) and minimum in loam (1.57 times over control). Similar trend of changes was observed in organic matter

(Table 5.3).

Table 5.3 Effect of green leaf powder amended in soil (@ 2%, w/w) of *A. conyzoides* on pH, electrical conductivity, organic carbon and organic matter in soils of different texture.

	Treatment	Clayey	Sandy	Loam	Clayey loam	Sandy loam
pH	Unamended	7.87^{c}	8.28^{b}	8.06^{d}	8.12^{c}	8.33^{a}
	Amended	7.67^{b}	8.10^{a}	7.57^{c}	7.50^{d}	7.62^{b}
EC (μS)	Unamended	149.1^{d}	111.1^{c}	169.6^{b}	288.5^{a}	158.1^{c}
	Amended	827.4^{d}	778.5^{c}	1043.5^{c}	1079.0^{b}	1196.5^{a}
OC (%)	Unamended	0.25^{a}	0.04^{c}	014^{b}	0.17^{b}	0.07^{c}
	Amended	0.48^{b}	0.45^{b}	0.22^{d}	0.57^{a}	0.03^{c}
OM (%)	Unamended	0.43^{a}	0.16^{d}	0.23^{c}	0.28^{b}	0.13^{d}
	Amended	0.83^{b}	0.78^{b}	0.34^{d}	0.98^{a}	0.52^{c}

EC: Electrical Conductivity; OC: Organic Carbon; OM: Organic Matter.
Similar superscripts in a row represent insignificant difference at $P<0.05$ applying DMRT.

Appreciable changes were also observed with respect to various nutrients *viz.* N, P, K, Na, Ca and Mg (Table 5.4). Available N was measured to be the maximum in clayey soil followed by clayey loam and loam. It was measured to be minimum in sandy soil i.e. 56.41 kg/ha. In all amended soils, content of available N increased than respective controls. Maximum increase was observed in loam followed by sandy soil and sandy loam. In clayey soil, the increase in N was only 1.25 times i.e. minimum compared to other soils. Further, K and P also differed with respect to different soil type *vis-a-vis* amendment. In unamended soil, maximum amount of P was observed in clayey soil. Similarly K was also the maximum in clayey soil. In sandy soils, however, least amount of both P and K was observed in umamended soils compared to others.The content of P in the amended soils increased by nearly 1.5 to 3 times.

Maximum increase of P was observed in sandy loam and minimum in clayey loam. In K maximum increase was observed in sand and minimum in clay.

Table 5.4 Effect of amendment of green leaf powder (2g/100g) of *A. conyzoides* on available macronutrient content in the soils of different texture.

	Treatment	Clayey	Sandy	Loam	Clayey loam	Sandy loam
N (kg/ha)	Unamended	71.43^{a}	56.41^{d}	65.83^{b}	69.20^{a}	60.27^{c}
	Amended	89.54^{b}	74.73^{e}	92.50^{a}	85.70^{c}	78.10^{d}
P (ppm)	Unamended	112.54^{b}	48.24^{e}	100.24^{b}	78.24^{c}	55.87^{d}
	Amended	150.24^{b}	129.03^{d}	150.20^{b}	142.38^{c}	173.20^{a}
K (ppm)	Unamended	193.33^{a}	66.67^{e}	106.67^{c}	133.0^{b}	86.67^{d}
	Amended	473.33^{c}	733.33^{a}	473.33^{c}	416.67d	580.0^{b}
Na (ppm)	Unamended	50.23^{b}	40.22^{d}	55.67^{a}	55.00^{a}	46.24^{c}
	Amended	69.24^{b}	64.27^{c}	68.40^{b}	74.23^{a}	65.00^{c}
Ca (g/100g)	Unamended	1.57^{a}	1.13^{d}	1.27^{b}	1.17^{c}	1.10^{c}
	Amended	4.70^{a}	2.67^{d}	4.10^{b}	4.10^{b}	2.85^{c}
Mg (g/100g)	Unamended	4.63^{a}	0.93^{d}	1.37^{c}	1.63^{b}	0.87^{c}
	Amended	8.03^{d}	2.47^{e}	2.10^{c}	9.23^{b}	9.95^{a}

Similar superscripts in a row represent insignificant difference at $P<0.05$ applying DMRT.

Like N, P and K, the contents of Na, Ca and Mg also increased in the amended soils (Table 5.4). Little change in Na in amended soil was observed with respect to different unamended soils. Maximum amount of Na was observed in clayey loam followed by loam and clay where as minimum was observed to be in sandy soils where it was about 40 ppm. It significantly increased in amended soil and maximum increase was observed in sandy soils (1.6 times over control) followed by sandy loam. In loam, however, magnitude of increase was the minimum (1.23 times over control). Like Na, the variation in content of Ca in various unamended soils was also less. However, in

amended soils, it increased significantly. Increase in Ca was the maximum in clayey loam followed by loam and clay. However, in sandy soils i.e. sand and sandy loam, increase in Ca was of lesser magnitude and it increased by only 2.4-2.6 times, respectively. The content of Mg also varied a lot among different types of unamended soil. Amount of Mg was the maximum in clayey soils and the minimum in sandy loam where its content was only 0.87 g/100g soil. In soil amended with leaf powder of *A. conyzoides,* the amount of Mg increased significantly (11 times over control) in sandy loam and loam. However, in sand and clay only soils, the increase in amount of Mg was lesser and it increased by 1.78 and 1.73 times, respectively (Table 5.4).

Further, the amount of total phenolics was also determined in amended and unamended soils (Fig. 5.3).The content of phenolics increased by many folds in amended soils, especially sandy and sandy loam soil. Minimum content of phenolics was found in clay (0.69 μ/g soil) and in amended soil, amount, was measured to be 3.46 μg/g soil, i.e. it increased 5 times. In sand, the increase in amount of total phenolics in amended soil was the maximum. Here, amount of phenolics in unamended sandy soil was 2.17 μ/g soil, whereas in amended soil it was 35.86 μ/g soil depicting 16.5 times increase (Fig. 5.3). Further, in sandy loam soil, the increase in amount of total phenolics was also high (8.7 times). In loam soil, the amount of phenolics in control was 7.13 and in contrast to amended soil, it was 16.78 μ/g soil, In clayey loam amount of phenolics in soil amended with 2% leaf powder was measured to be 8.39 μ/g soil which exhibited 4.6 times increase than control. Thus maximum increase in amount of phenolics was observed in sandy soils whereas it was minimum in loam (Fig. 5.3).

Discussion

In the present study, the early growth of *O. sativa* was observed to be relatively better in sandy soils (unamended control conditions) with alkaline pH. This was followed by clayey soil. Both clayey and sandy soils with pH values near 8 are reported to be suitable for *O. sativa* germination and early growth (Brady, 2003; Handbook of Agriculture, 2003). In other words, *O. sativa* can grow well both in sandy and clay soils, as also observed in the present study. When such soils were amended, a clear cut influence of texture could be seen on the rice growth. Magnitude of inhibition of *O. sativa* seedlings was less in loam, clayey loam as well as clay soil compared to sandy soils i.e. sand only and sandy loam. In the sandy soil, rate of inhibition was the maximum followed by sandy loam. In these soils nearly 92 % and 87% (respectively) inhibition of seedling length was observed when amended with 2% of *A. conyzoides* leaf powder. This shows that phytotoxicity is influenced by soil texture. Similar observations were also made by other workers who indicated that soil texture influences allelopathic effect of plants (El-Darler and Youssef, 2000; Einhellig, 1996).

The phytotoxic influence of amended soil was directly related to the amount of phenolics present i.e. maximum phenolics were found in sandy soil where maximum inhibitory effect was seen. In sandy soils, maximum amount of phenolics could be due to their non adsorption to soil particles and thus direct availability for causing inhibition. In other soils, however, due to adsorption to soil particles, the phenolics may not be directly available to plant system and thus caused less inhibition.

In order to find whether phenolics influence *O. sativa* growth directly or indirectly through the influence on soil properties, the amended and unamended soils

were analyzed for nutrients and some chemical properties. The result indicated that amount of nutrients was more in the amended soils (irrespective of texture) and hence were not limiting to the growth of *O. sativa* seedlings. Nevertheless, phenolics may interfere in the uptake of nutrients by the growing seedlings. There are some other reports also, that indicate the interference of phenolics in the uptake of nutrients by the plants (Bazirамekenga *et al.,* 1994; Kaushal *et al.,* 2006; Khan *et al.,* 2006; Minghua *et al.,* 2007). However, there is no direct evidence for this.

Based on these observations, it can be concluded that phytotoxicity of *A. conyzoides* is significantly influenced by the soil texture.

EXPERIMENT – 6

Objective

To study the phytotoxic effect of roots of *Ageratum conyzoides* on some test plants

Hypothesis to be tested

Since roots are the only part of the plant, which are in direct contact with soil, their contribution in imparting the allelopathic effect can not be ignored. The present experiment was therefore designed to assess:

- If roots contribute towards phytotoxicity of *A. conyzoides*?
- The nature of allelochemicals and how they are released?
- If so, what is their dynamics of release?
- Whether ploughing of fields after cutting the above ground part of plant alters its phytotoxicity.

Parameters Studied

Growth of test plants was studied in terms of seedling length and seedling dry weight in response to root extracts and in soil amended with fresh or dried roots of *A. conyzoides.* Changes in amount of phenolics, their dynamics of release with time and available macro- and micronutrients in *A. conyzoides* soil were also determined.

Methodology

Roots of *A. conyzoides* were separated from the freshly collected plants (at flowering stage) growing in the area around the campus of Aligarh Muslim University, Aligarh. One half of these collected roots were used as fresh material while the remaining was shade-dried, powdered and stored in labeled polyethylene

bags for further use. Healthy and uniform seeds of *Anagalis arvensis*, *Brassica oleracea* var. *botrytis*, *Cicer arietinum*, *Phaseolus mungo* and *Oryza sativa* procured from National Research Centre for Weed Science, Jabalpur (M.P.) and Indian Agricultural Research Institute (IARI), New Delhi were used as test plants.

Aqueous root extracts (0.5, 1 and 2%; w/v) were prepared as per details given in chapter material and methods. The pH of extracts varied from 6.82 to 6.98, whereas conductivity ranged from 610 μS to 2.7 mS. For experiment purposes, soil was amended as under:

a) 500 ml of root extracts of each concentration were directly amended in soils.

b) 0.5, 1 and 2 g of root powder was mixed per 100 g soil. These were thoroughly mixed and used for growth studies.

c) Fresh chopped roots were incorporated into the soil at the rate of 5, 10 and 20 g / kg soil and mixed with 300 ml of pure water. These were kept for 24, 48 or 72 h. Seeds of *C. arietinum* were sown in these amended soils.

For all amendments and for each concentration as well as each test plant, five replicates were maintained along with a set of unamended (control) soil. Amounts of phenolics in extracts, and root powder amended soils were determined as per method of Swain and Hillis (1959). Besides this, dynamics of release of phenolics with time was also studied in fresh root amended soil. Changes in various available nutrients in root powder amended soils were also determined as per methods given in materials and methods. All the experiments were repeated and mean of all data was subjected to one-way analysis of variance.

Results

a) Growth Studies in Extracts

A steady decrease in seedling length of all test plants was noticed in response to root extracts of *A. conyzoides* (Fig. 6.1a). In *Anagalis arvensis,* the seedling length decreased by nearly 38% in samples treated with 1% concentration (9.55±0.78 cm), and 62% in those receiving 2% extract treatment. In *Brassica oleracea* var. *botrytis,* seedling length was 16.55±0.55 cm in control whereas at 2% extract concentration, it was only 7.58±0.46 cm indicating a reduction of nearly 54%. Likewise, in *C. arietinum* seedling length was significantly reduced by 39% and 68% in response to 1% and 2% extract, respectively, compared to control. Almost similar trend of decrease was observed in seedling length of *P. mungo* and *O. sativa.* In response to 2% concentration of extract, seedling length in both the test plants was reduced by nearly 55% compared to control (Fig. 6.1a). The values of correlation coefficient were significant and greater than -0.9.

Seedling dry weight also decreased as the extract concentration increased (Fig. 6.1b). In *Anagalis arvensis* it was nearly 5.89±0.12 mg in control. It was found to decrease, though insignificantly, at lower concentration. However, at *2%* concentration of extract, a significant reduction (57%) was observed where seedling dry weight was only 2.5±0.1 mg. In *Brassica oleracea* var. *botrytis*, dry weight in control was 11.64±0.62 mg and it was reduced by around 49% in response to 2% concentration. Likewise, in *C. arietinum* a concentration dependent decrease was seen and dry weights decreased by 15 and 34% respectively, in those treated with 0.5 and 1% root extracts compared to control. However, at 2% concentration, the dry weight was reduced by nearly 55%. Likewise in *P. mungo* dry weight decreased with the

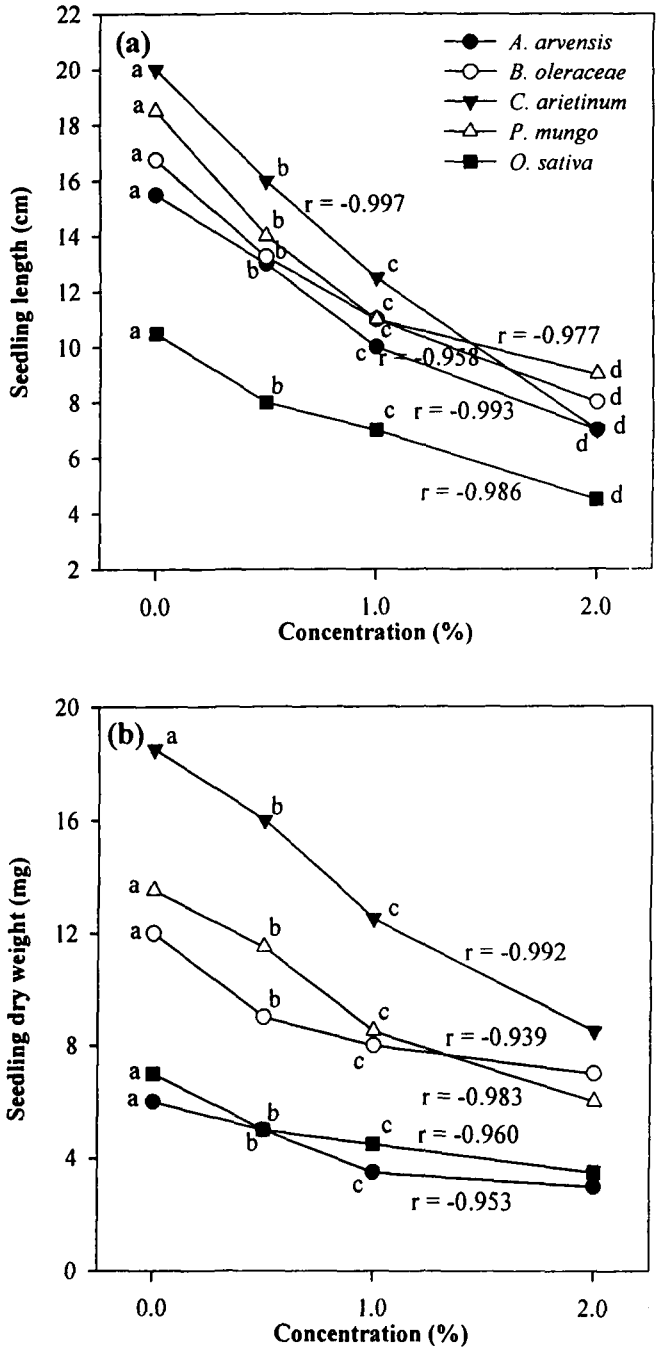
every increase in concentration of root extract. It was only 5.24±1.0 mg at 2% concentration compared to 13.25±0.78 mg in control. In *O. sativa* samples treated with 1% extract seedling dry weight decreased insignificantly. With 2% treatment of extracts, it was 2.95±0.05 mg as compared to control, where seedling dry weight was 6.99±0.25 mg (Fig. 6.1b). In each of the test plants, a strong correlation coefficient was observed between values of seedling dry weight and concentrations of root extracts (Fig. 6.1b).

b) Growth Studies in Amended Soil

To study the effect of root extracts in soil, test plants were grown in soil, incorporated with (or without in case of control) different concentration of root extracts. Here also, the seedling length of different test plants significantly decreased in all types of root extract amended soils. In *Anagalis arvensis* seedling length in control was 16.1±0.37 cm whereas in soil amended with 2% root extract, it was reduced by 48.8% (Fig. 6.2a). In *Brassica oleracea* var. *botrytis*, seedling length in unamended soil was 18.94±0.5 cm. It decreased by around 47% in soil amended with 2% root extracts. However, in *C. arietinum*, seedling length was almost half of that of control in samples where soil was amended with 2% root extracts. As in other test plants, an appreciable decrease of nearly 44% was noticed in seedling lengths of *P. mungo* and *O. sativa.*

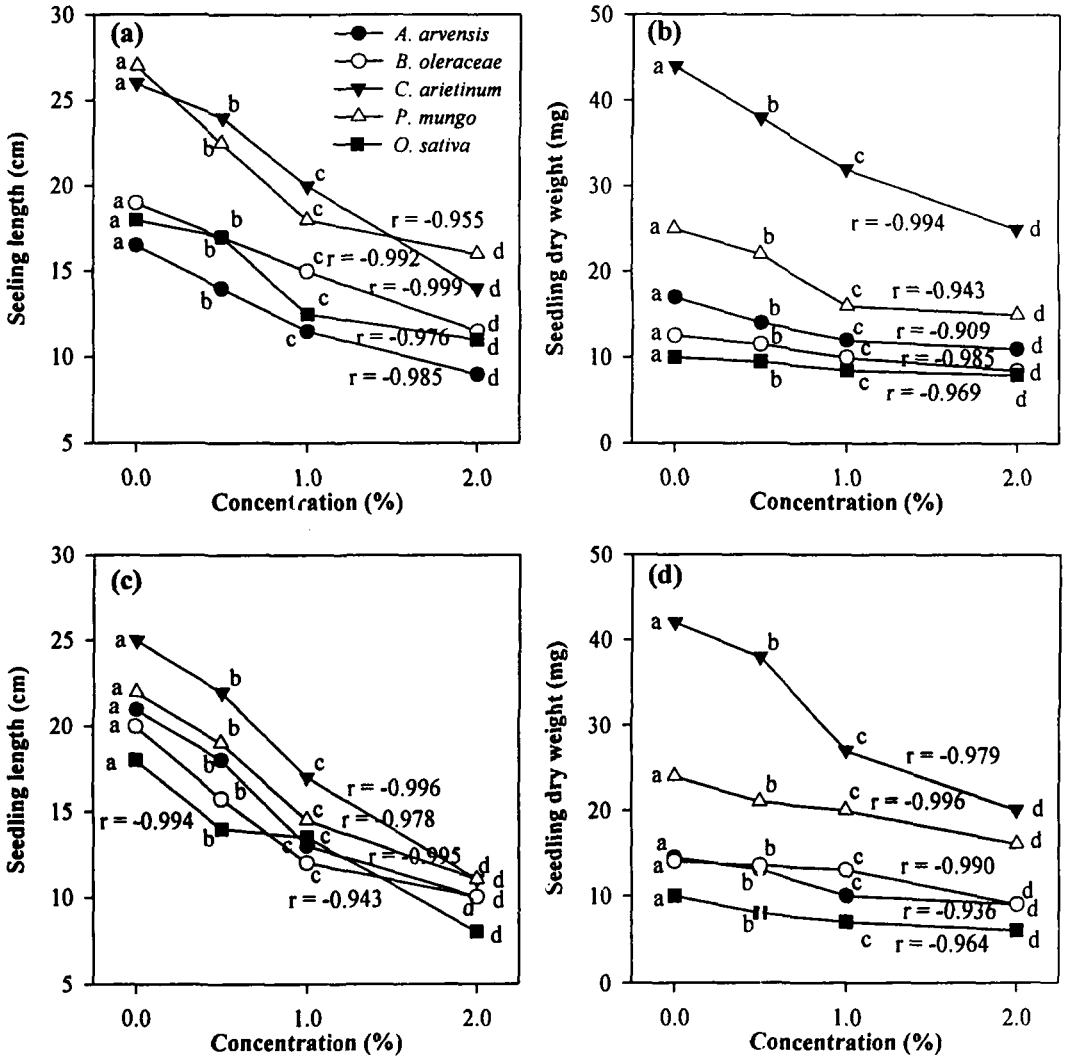
Almost similar trend of reduction was observed in dry weights of test plants grown in extract amended soils. In *Anagalis arvensis* and *Brassica oleracea* var. *botrytis,* seedling dry weight was reduced by 44.65 and 43.4%, respectively, in 2% extract amended soil (Fig. 6.2b). In soil amended with 2% root extract, seedling dry weight of *C. arietinum* was 22.36±0.78 compared to 43.25±1.92 mg in control

Fig. 6.1. Effect of different concentrations of root extracts of *A. conyzoides* on (a) seedling length (b) seedling dry weight of different test plants.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Fig. 6.2. Effect of different concentrations of root extracts (a), (b) and root powder (c), (d) of *A. conyzoides* when amended in soil on seedling length and seedling dry weight of test plants.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

(Fig. 6.2b). Likewise, in *P. mungo* and *O. sativa* also, significant decrease in dry weight was noticed and it decreased with every increasing concentration. More inhibition was observed in *P. mungo* (45.65%) than *O. sativa* (38.13%), when compared to their respective controls (24.84±0.54 and 10.15±0.31 mg, respectively, Fig. 6.2b).

Growth studies were also conducted in soils amended with root powder.

Growth of each test plants in amended soils was less compared to their respective controls. In *Anagalis orvensis* and *Brassica oleracea* var. *botrytis*, the seedling length was reduced by nearly 52% in soil amended with 2% root tissue compared to unamended control (Fig. 6.2c). In contrast, in *C. arietinum*, a reduction of nearly 55% in dry weight was observed at the same concentration. Almost similar trends of reduction were also observed in *P. mungo* and in *O. sativa* where seedling length was reduced by nearly 50 and 52% respectively (Fig 6.2c). As regards dry weight, comparatively more reduction was noticed in *Anagalis arvensis* than *Brassica oleracea* var. *botrytis* in case of higher concentration where seedling lengths were 7.6±0.78 and 7.68±0.31 mg compared to their respective controls (15.1±0.26 and 14.68±0.25 mg, respectively). However, in *C. arietinum*, dry weight of seedlings from soil amended with 2 g powder was reduced by around 58.78% (Fig. 6.2d). In *P. mungo* and *O. sativa* the magnitude of reduction was less at lower concentration (1%) whereas at highest concentration, a significant reduction of 42.86 and 49.9% was observed compared to their respective controls (Fig. 6.2d). A significant and strong reciprocal correlation was calculated between the growth of test plants in amended soils and concentration of amendments.

c) Determination of Soil Properties

Since retardatory effect on growth was more in samples containing root powder, these soils were subjected to nutrient analysis so as to find out whether there is any change in nutrient status or not. The pH of soil amended with root powder was lesser than that of control and it varied from 7.48 to 7.35. However, a reverse trend was observed in terms of conductivity (from 531 to 1699.33 μS) and it was less in the unamended control soil compared to root powder amended soil. Likewise, amount of

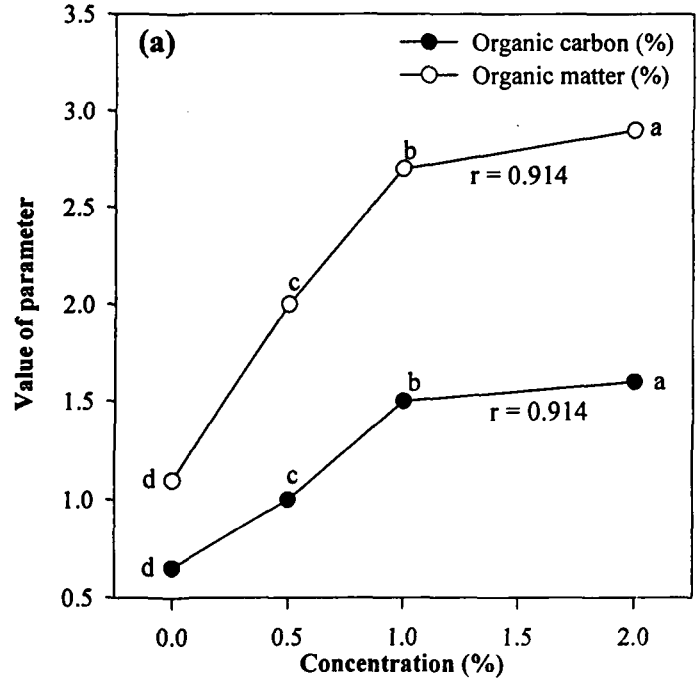
organic carbon (OC) and organic matter (OM) was also more in amended soils. It was 2.7 times more in soil amended with 2 g root powder than that of control (Fig. 6.3a).

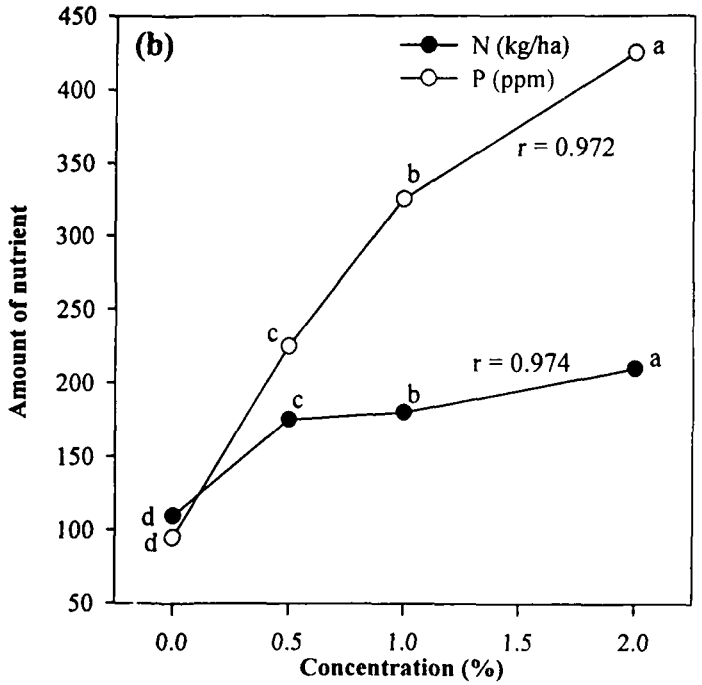
Even the amount of available nutrients was more in amended soil compared to unamended control, though to variable extent. Amount of N. increased by 2.27 times (over that of control where its amount was 112.2 kg/ha) in soil amended with 2 g powder (Fig. 6.3b). Likewise, amount of P increased significantly in amended soils even at lower concentrations i.e. 0.5 and 1 g of amendment. Here, it was nearly 2.3 and 4.0 times more than control, respectively (Fig. 6.3b). However, with 2 g amendments, it increased by around 4.5 times (428 ppm) over control (95.3 ppm).

Almost similar trend of increase was noticed in content of K and Na, (Fig. 6.4a) where their amounts increased with increasing concentration of amendments. At highest concentration, amount of K increased by 6.5 times (1490 ppm) whereas that of Na increased by nearly 2 times (152.5 ppm) of that of control (82.5 and 230 ppm, respectively). Likewise, the amount of Ca and Mg also increased significantly in the amended soil. In 2 g powder amended soil, available Ca and Mg increased by nearly 3.8 and 2.6 times, Respectively, over control (8.33 and 5.53 g/100g soil, respectively; Fig. 6.4b). Almost similar magnitude of increase i.e. 2.36 and 2.75 times that of control was observed in Cl and HCO_3, respectively (Fig. 6.4b).

Like macronutrients, amount of micronutrients also increased with the increase in concentration of amendment (except Fe). However, at lower concentration i.e. 0.5 and 1 g root powder amended soil, the increase was comparatively less. Amount of Fe and Mn, increased in the amended soils and at 2% concentration, it was1.75 and 1.32 times of that in control, respectively (Fig. 6.4c), Almost similar

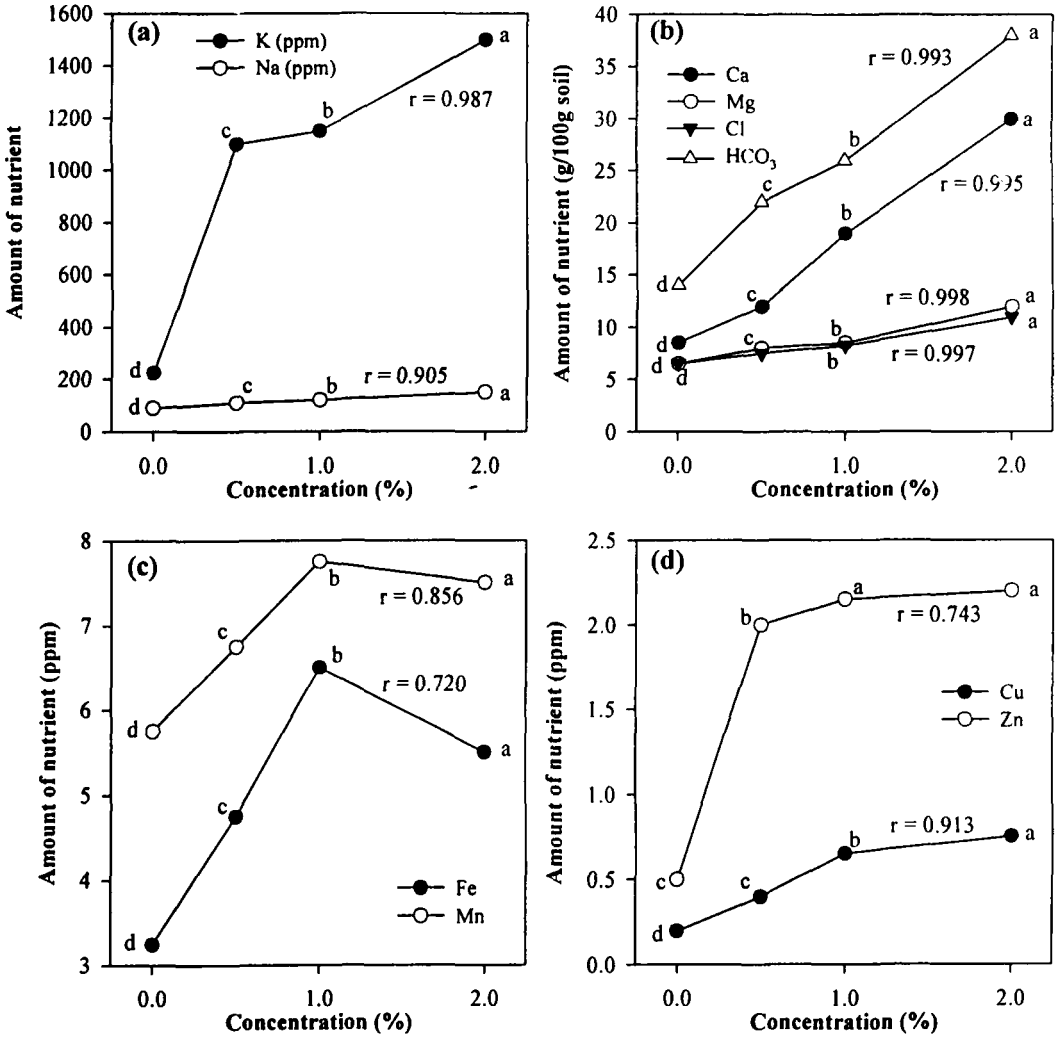
Fig. 6.3. Changes in amount of (a) organic content (b) nitrogen and phosphorus in soil after amendment of root powder of *A. conyzoides*.





Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

Fig. 6.4. Changes in amount of available nutrients in soil after amendment of root powder of *A. conyzoides* in soil.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

trend was observed in amounts of Cu and Zn, which were 0.75 and 2.16 ppm in 2 g powder amended soil indicating an increase of 3.4 and 4.4 times, respectively, compared to control (Fig.6.4d).

d) Estimation of Total Phenolics

Root extract as well as root powder amended soils were analyzed for the water-soluble allelochemicals - phenolics. Aqueous extracts as well as powder amended soils were found to contain an appreciable amount of phenolics and their

amount increased with increasing concentration.

In 0.5% root extracts amount of phenolics was found to be 1.104±1.08 μg/ml, whereas it increased to 139.62±1.99 μg/ml in 1% extracts. However, at highest concentration i.e. 2% of root extracts total amount of phenolics was measured to be 293.24±0.92 μg/ml and was nearly double than that in 1% root extracts (Fig. 6.5a).

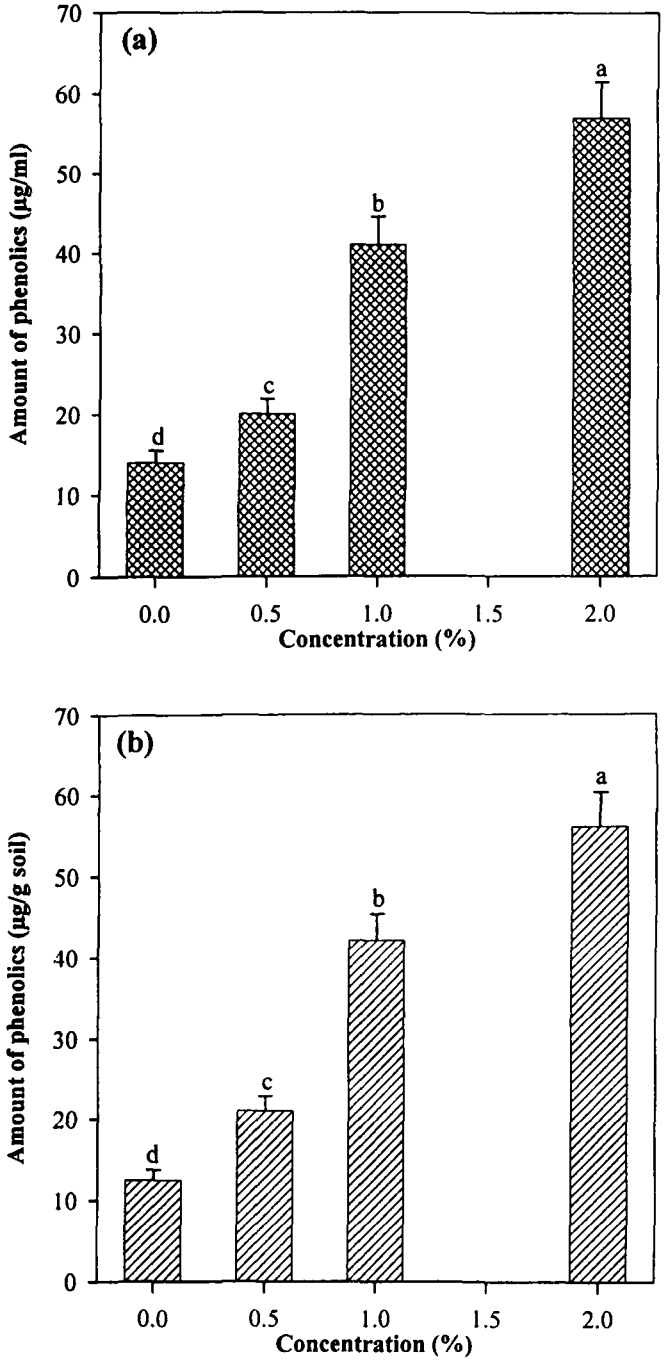
Comparatively, lesser amount of phenolics was assessed in powder amended soils compared to that of aqueous extracts of same concentration. However, it was more than that of unamended control soil. With the amendment of 0.5 and 1g root powder, their amount increased by 2.43 and 3.48 times, respectively. However, maximum increase of 4.19 times was observed when soil was amended with 2 g root powder (Fig.6.5b).

Besides the changes in amount of phenolics upon amendments, the dynamics of release of phenolics with time was also studied in soil incorporated with fresh chopped roots. Fresh roots were incorporated into soil and were kept for different time period ranging from 1 to 120 h. The amount of phenolics increased gradually with increase in time up to 24 h where it was maximum (25.96 μg/g; Fig. 6.6) However, the amount decreased after 36 h and was estimated to be 20.99 μg/g soil, Further, at 72 h, the amount again increased to 22.52 μg/g soil (Fig. 6.6).

(e) Growth Studies with Fresh Roots

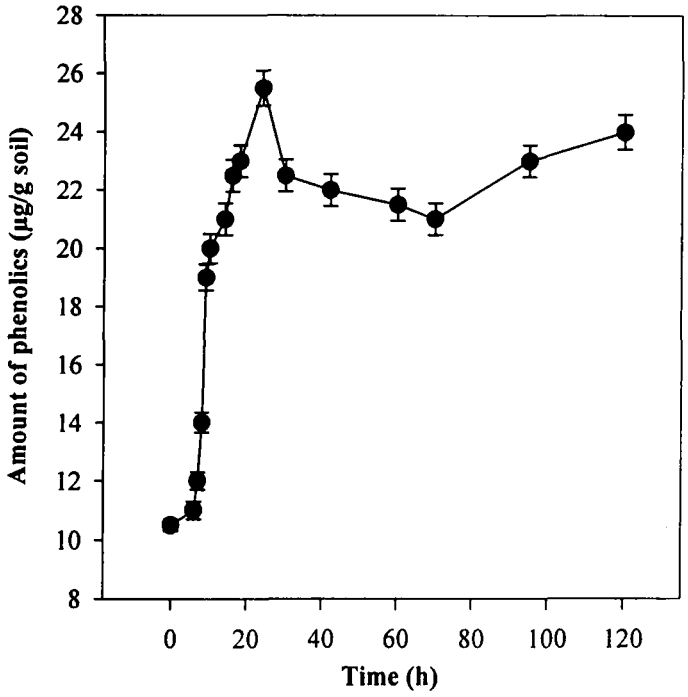
Since roots contained an appreciable amount of phenolics and their powder or extracts were inhibitory in nature another experiment was performed with fresh chopped roots. Growth studies were conducted in soil mixed with 5, 10 and 20 g fresh chopped roots or without roots (control) and water. Seeds of *C. arietinum* were sown

Fig. 6.5. Amount of total phenolics estimated in (a) aqueous extracts of roots of *A. conyzoides* and (b) soils amended with root powder.



Different alphabets along a curve represent significant difference at $P<0.05$

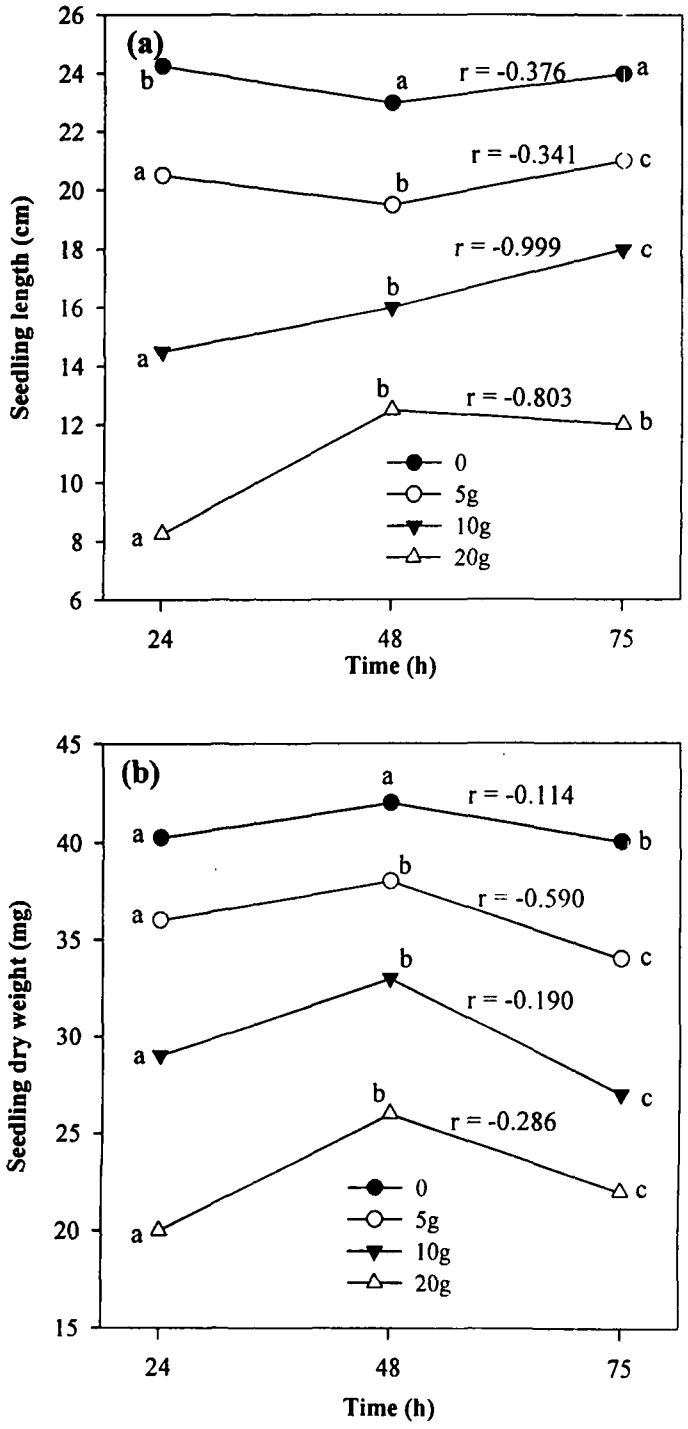
Fig. 6.6. Dynamics of release of phenolics (±SD) from root of *A. conyzoides* (amended in soil) with time.



in these soils after 24, 48 and 72 h of mixing. As in case of soil amended with root powder and root extracts, the seedling length of *C. arietinum* was shorter in those grown in soil mixed with 5, 10 and 20 g fresh chopped roots of *A. conyzoides* compared to control (Fig. 6.7a). A significant reduction was observed even at the lower concentrations. At highest concentration (20 g/kg soil), the seedling length was reduced by nearly 50%.

Similar observations were made in case of soils with similar amount of roots but kept for 48 and 72 h. In soils, where roots were kept for 48 h, nearly 40% reduction over that of control was seen in case of *C. arietinum* grown in soil incorporated with highest amount of fresh roots. Further, the seedling length of *C. arietinum* grown in soil after 72 h of mixing was reduced by 45%. At highest rate of amendment, maximum reduction in seedling Length was observed when amended

Fig. 6.7. Effect of fresh roots (amended in soil) of *A. conyzoides* on (a) seedling length and (b) seedling dry weight of *C. arietinum* with time.



Different alphabets along a curve represent significant difference at $P<0.05$; r represents value of correlation coefficient

soils were kept for 24 h followed by 72 and 48 h, respectively (Fig. 6.7a).

A similar trend of reduction was observed with respect to dry weight in all types fresh chopped root amended soils. Maximum inhibition (48.9%) was observed at highest concentration in the soil kept for 24 h (Fig. 6.7b), whereas, at the same concentration, in soil kept for 48 and 72 h, the magnitude of reduction was 36.77 and 44.04%, respectively (Fig. 6.7b).

Thus, from the above results, it is clear that roots of *A. conyzoides* exhibit allelopathy towards test plants. These contained phytotoxic phenolics in them and the release of phenolics under normal conditions gradually increases with time up to 24 h and thereafter it decreases.

Discussion

In the rhizosphere environment, root exudates represent one of the largest inputs of plant allelochemicals. The present study highlights the phytotoxic influence of fresh and dried roots of *A. conyzoides* on other test plants. Roots, in fact, are those parts of plant that are directly in contact with soil and hence play an important role in imparting phytotoxicity to plants. The pattern of rooting varies from plant to plant and is also influenced by type of soil and interactions between roots and soil (Uren, 2000). In *A. conyzoides,* the root system is shallow and hardly exceeds 20 cm depth with a number of secondary and tertiary roots that remain within the top 10 cm depth. It spreads around 400-425 cm^2 in rhizosphere. It may thus affect the growth and establishment of test plants.Both fresh roots or dried roots may influence growth of other crops. This is indicated in the present study also, where extracts prepared from root powder significantly inhibited growth of *Anagalis arvensis*, *Brassica oleracea* var. *botrytis*, *Cicer arietinum*, *Phaseolus mungo* and *Oryza sativa.* However, the phytotoxicity differs with different species depending upon their susceptibility

towards extracts. Not only root extract but also extract and root powder amended soils exhibited phytotoxicity. The soils amended with root powder were more phytotoxic than extract amended soils. Several other studies have also shown the inhibitory effect of extract and residue of root towards test plant (Kazinczi *et al.*, 1999; Kiemnec and McInnis, 2002; Kong *et al.*, 2006; Haibin *et al.*, 2007; Koloren, 2007; Sisodia and Siddiqui, 2007a). Like the phytotoxic effect of dried roots, experiments were also conducted with the fresh chopped roots. This was considered significant because root damage alters the rate of exudations in the soil. Nardi *et al.*, (2000) reported that roots inhibited the growth of other plants and change the chemical and physical properties of soil through exudation. The phytotoxic effect of such soils was established towards *C. arietinum.* Some interesting results were obtained in this case. The growth of *C. arietinum* was inhibited when fresh roots were allowed to remain in soil for 24 h, thereafter, it declined and decreased significantly. After 72 h, however, the inhibition of *C. arietinum* was more than 48 h but lesser than that of 24 h. That means phytotoxicity of fresh roots was the maximum when these were placed for 24 h in soil medium followed by those where amendment of fresh roots was done for 72 h. Least phytotoxicity was observed when amendment was done for 48 h. Amount of phenolics - the well known allelochemicals, was also determined at different time intervals and it correlated well with pattern of phytotoxicity. This shows that amount of phenolics increases twice when roots were amended in soil. The first maximum increase was observed at 24 h and the second at 72 h. Such a pattern of allelochemicals showing two maxima is not the first report as it has been earlier reported in *Vulpia* (An *et al.*, 1996). This clearly indicates that roots (both fresh and dried) actively participate in allelopathic interactions and bear great significance in

root-mediated interactions in the rhizosphere.

The study concludes that the large amount of roots that remain in soil after removal of above ground parts of the weed also inhibit the growth of test plants. However, ploughing may play important role in their removal and displacement and thus may help in reducing the allelopathic potential of roots of *A. conyzoides*.

EXPERIMENT – 7

Objective

To study the periodic changes in phytotoxicity of leaf or root residues of *Ageratum conyzoides* during decomposition process.

Hypothesis to be tested

Phytotoxicity of the plant residues varies greatly during decomposition process, depending upon the medium and the prevailing environmental conditions. Moreover, allelochemicals released by plant into environment undergo various changes over time or with varying environmental conditions. The experiment was designed to determine the changes/alterations in phytotoxicity of *A. conyzoides* residues either alone or when mixed in soils during decomposition process.

Parameters Studied

Growth of *Brassica oleracea* var. *botrytis* (in terms of seedling length and seedling dry weight) in response to residue extracts alone or mixed in soil was measured at different time intervals. In addition, changes in pH, electrical conductivity and amount of total phenolics were also determined.

Methodology

Experimental Design

For this experiment decomposition of leaves (forming bulk of above-ground parts) and roots (below-ground parts) was studied. Leaf or root powders (hereafter referred to as residues) either alone or in mixtures with soil was studied for 60 days. Four different combinations / treatments of root or leaf residues of *A. conyzoides* were prepared as under:

- Residue of leaves or roots alone (hereafter referred to as R)
- Soil alone (hereafter referred to as S)
- Residue and soil in the ratio of 1:1 (w/w) (hereafter referred as R+S)
- Residue and soil in the ratio of 1:3 (w/w) (hereafter referred as R+3S)

For residues only (R), 80 ml of pure water was mixed well in 20 g of each residue in a 500-ml glass jar with a lid. For soil (S), mixing was done by taking 20 g soil with 5 ml water in a 500-ml glass jar. While in treatments of mixtures of residues and soil, mixing was done by adding 50 and 30 ml of water in 20 g mixture of each R+S and R+3S, respectively.

Each treatment was allowed to decompose for two months by incubating them in dark at 20°C. Studies on the phytotoxicity and other characteristics were done after 1, 5, 10, 15, 20, 25, 30, 45 and 60 days. In each jar, small holes of 2 mm diameter were made in lids for exchange of air. In all treatments, moisture content was kept constant by daily weighing the jars and supplementing the evaporated water loss.

Preparation of Aqueous Extracts

For each treatment, i.e. R, S, R+S and R+3S, aqueous extracts were prepared after 1, 5, 10, 15, 20, 30, 45 and 60 days by adding 480 ml, 437 ml, 481 ml, and 453 ml of pure water, respectively (so as to recover about 500 ml extract) and stirred for 30 min on rotary shaker and allowed them to settle for 16-18 h. The mixture thus obtained was filtered through 2 folds of muslin cloth, centrifuged at 3500 rpm for 30 min and then filtered through Whatman no. 1 filter paper. These extracts were used for further studies.

Growth Studies in Extracts

Growth studies with extracts under laboratory conditions were carried in

similar manner as explained in chapter material and methods. Seeds of *Brassica oleracea* var. *botrytis* (used as test plant) were allowed to germinate in water or each treatment of leaves or roots. After eight days, seedling lengths and seedling dry weights were measured.

Determination of pH, Conductivity and Total Phenolics of Extracts

Changes in pH and conductivity of extracts obtained at different periods of decomposition were determined with the help of digital pH and conductivity meters, respectively. Estimation of total phenolics was done following method given by Lowe (1993).

Procedure

For the estimation of total phenolic content, 0.5, 1.0, 2 and 5 ml extracts of R, S, R+S and R+3S treatments, respectively, were taken in labeled test tubes. The final volume was made to 10 ml (wherever required) with pure water. Along with these, 10 ml of 25 ppm ferulic acid (as standard) or 10 ml of pure water (as blank) were also taken. For each treatment (extract / standard / blank), five replicates were maintained. To each test tube, 3 ml of 20% Na_2CO_3 solution (w/v) followed by 1 ml Folin-Ciocalteau reagent were added. The solutions were mixed well and allowed to stand for 1 h at room temperature (20-25 °C). After 1 h, the absorbance was read at 700 nm in spectrophotometer against pure water blank. The total phenolic contents were calculated against ferulic acid as μg equivalents per ml of extract.

Statistical Analysis

The whole experiment was repeated with five replicates each time and mean values were used for statistical analysis using one-way ANOVA followed by

separation of means using Duncan's multiple range test and significance was checked at $P< 0.05$.

Results

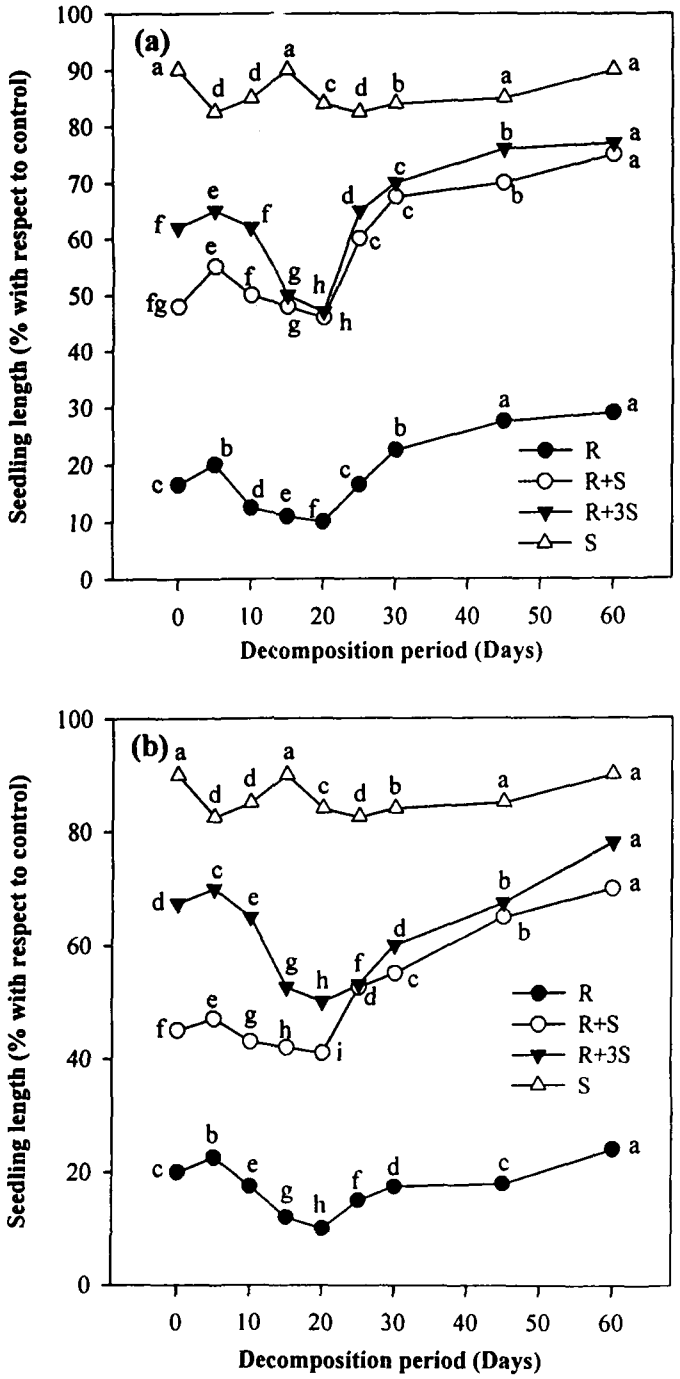
(a) Effect on Growth of *Brassica oleracea* var. *botrytis*

The seedling lengths of *Brassica oleracea* var. *botrytis* in extracts of residues of leaves and roots, kept for one-day of decomposition period, were measured to be 16.56 and 20.27%, with respect to control, respectively (Fig. 7.1a,b, respectively). Compared to this, the value of seedling length was observed to be significantly more when grown in extracts prepared from residue decomposed for 5-days. After five days, the seedling length in extracts of leaf or root residues decreased significantly till 20-days where it was measured to be the minimum. At this period of decomposition, seedling length was measured to be nearly 10% of that of control (Figs. 7.1 a, 7.1b).

As the decomposition period proceeded further, there was an increase in seedling length of *Brassica oleracea* var. *botrytis*. However, comparatively lesser inhibition was observed when these residues were mixed in soil. In such treatments, seedling length of *Brassica oleracea* var. *botrytis* increased significantly by the end of decomposition and magnitude of increase was more than those in extracts of residues alone. In R+S treatment of roots, the seedling length in extract of 1-day decomposition was 49.7% of control whereas in leaves it was around 45% of control (Fig. 7.1 a, b).

In each part whether leaves or roots, as the decomposition continued the inhibition increased significantly and maximum inhibition was measured in extracts of 20-day-old decomposed residues. This is indicated by lesser values of seedling length of *Brassica oleracea* var. *botrytis* with respect to control. At this stage of

Fig. 7.1. Changes in seedling length of *B. oleraceae* with time during decomposition of (a) leaves and (b) roots of *A. conyzoides*.



Different alphabets along a curve represent significant difference at $P<0.05$

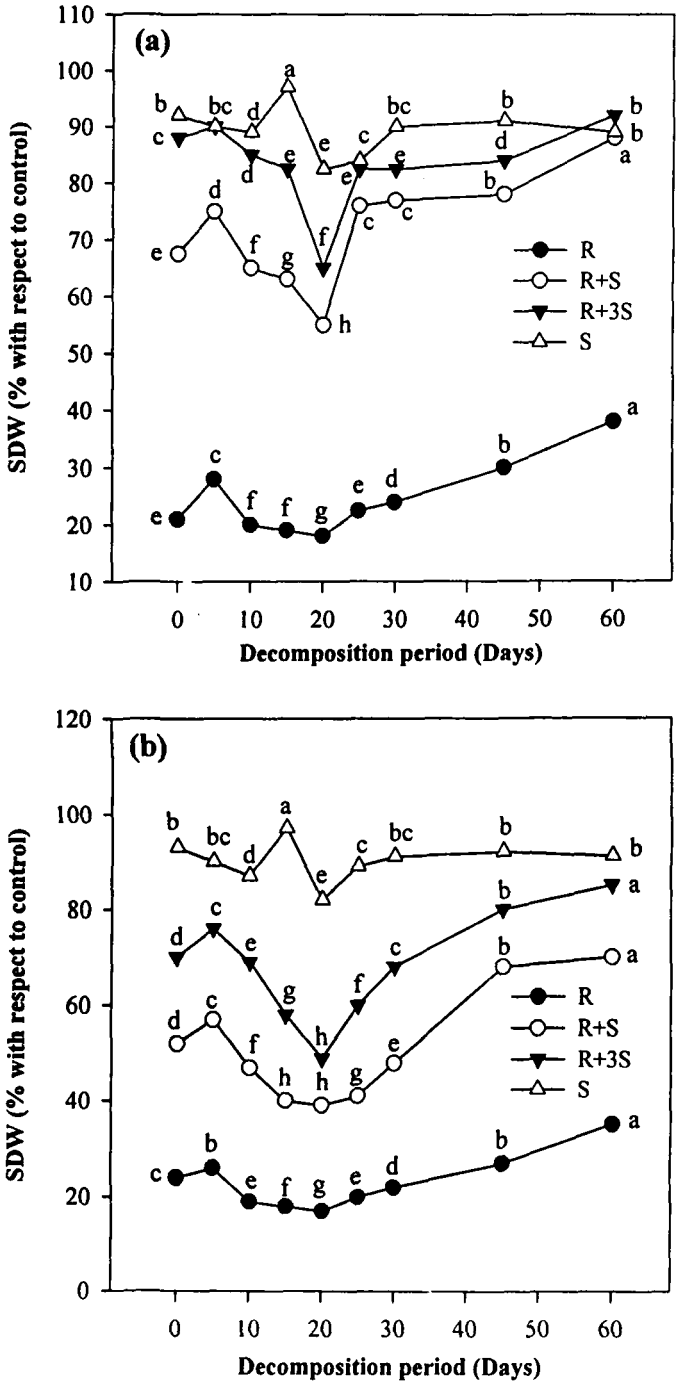
decomposition, the percent values of seedling lengths in root extracts were found to be lesser (36.55% with respect to control) than leaf extract (46.10% with respect to

control). Almost a similar trend of result was observed at the end of decomposition period, i.e. after 60-days, where seedling length of *Brassica oleracea* var. *botrytis* in root and leaf extract was measured to be nearly 80% and 78.5%, respectively, compared to control (Fig. 7.1a,b).

Almost similar pattern of reduction was observed when growth studies were carried with R+3S treatment. The inhibition in seedling length eventually increased as decomposition proceeded. The seedling length was measured to be nearly 50% with respect to control each in leaves or roots (Fig. 7.1a, b). Here also, the inhibition was lesser during later stages of decomposition. The inhibition in seedling length of *Brassica oleracea* var. *botrytis* treated with extracts made after 60-days of decomposition period was almost negligible. At this stage seedling length was observed to be nearly 85% with respect to control was both in leaves or root extracts decomposed for 60 days. In case of soil, however, the seedling length increased or decreased insignificantly with respect to control during the whole decomposition period (Fig. 7.1a, b).

Similar observations were made with respect to seedling dry weight (Fig. 7.2a, b). Here also, minimum value of seedling dry weight was observed in extracts of residues irrespective of leaf or root. It was followed by soil and residue mixture R+S and R+3S and maximum value of seedling dry weights were observed in soil only extracts where it varied insignificantly from those of control. The trend of changes in seedling dry weight with time was almost same as seen in case of seedling length (Fig. 7.2a, b).

Fig. 7.2. Changes in seedling dry weight of *B. oleraceae* with time during decomposition of (a) leaves and (b) roots of *A. conyzoides*.



Different alphabets along a curve represent significant difference at $P<0.05$

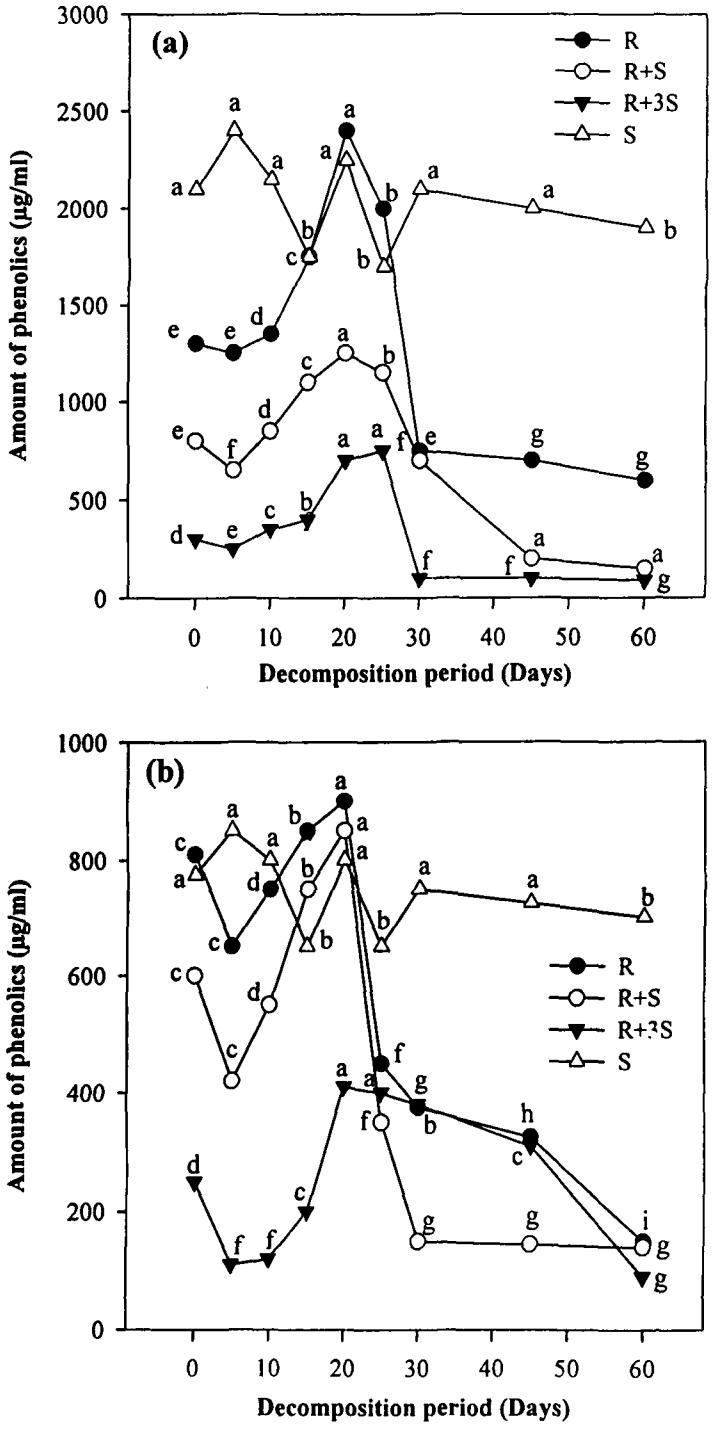
b) Changes in Total Phenolic Content

In general, amount of total phenolics was found to be the maximum in extracts

of residue alone at any period of decomposition and irrespective of part. This was followed by residue mixed soil treatments (i.e. R+S and R+3S). Least amount of phenolics was, however, estimated in soil (alone) extracts. Amount of phenolics increased significantly during early stages of decomposition and was the maximum after 20 days of decomposition. However, during end stages of decomposition, the phenolic content decreased significantly. In leaf residue extracts, the amount of phenolics was measured to be 1279.91±5.27 μg/ml after 1 day of decomposition (Fig. 7.3a). As decomposition proceeded, it increased significantly up to 20 days and measured to be the maximum i.e.2408.33±14.86 μg/ml. By the end of decomposition, only 483.60±9.20 μg/ml of phenolics was estimated. In extracts of R+S treatment, maximum content of phenolics was estimated to be 1266.76±4.91 μg/ml whereas in R+3S, maximum content was 642.83±6.23 μg/ml on 20^{th} and 25^{th} day, respectively (Fig. 7.3a).

Almost similar trend was observed in root residue extracts. Amount of phenolics was estimated to be 809.83±9.58 μg/ml in extracts after one day decomposition (Fig. 7.3b). However, this increased significantly up to 20 days and was measured to be 918.41±2.73 μg/ml. Thereafter, it declined with increasing decomposition period (Fig. 7.3b). In case of R+S treatment, a similar pattern of dynamics of phenolics was observed (Fig. 7.3b). Extracts of treatments decomposed for 20 days contained maximum phenolics (854.49±14.98 μg/ml) followed by those decomposed for 15 days (731.67±6.60 μg/ml). On the other hand, in R+3S treatment maximum value (415.0±4.40 μg/ml) of phenolics was observed on 20^{th} day of decomposition (Fig. 7.3b). However, in extracts of soil alone, the phenolic content

Fig. 7.3. Changes in total phenolic content with time during decomposition of (a) leaves or (b) roots of *A. conyzoides*.



Different alphabets along a curve represent significant difference at $P<0.05$

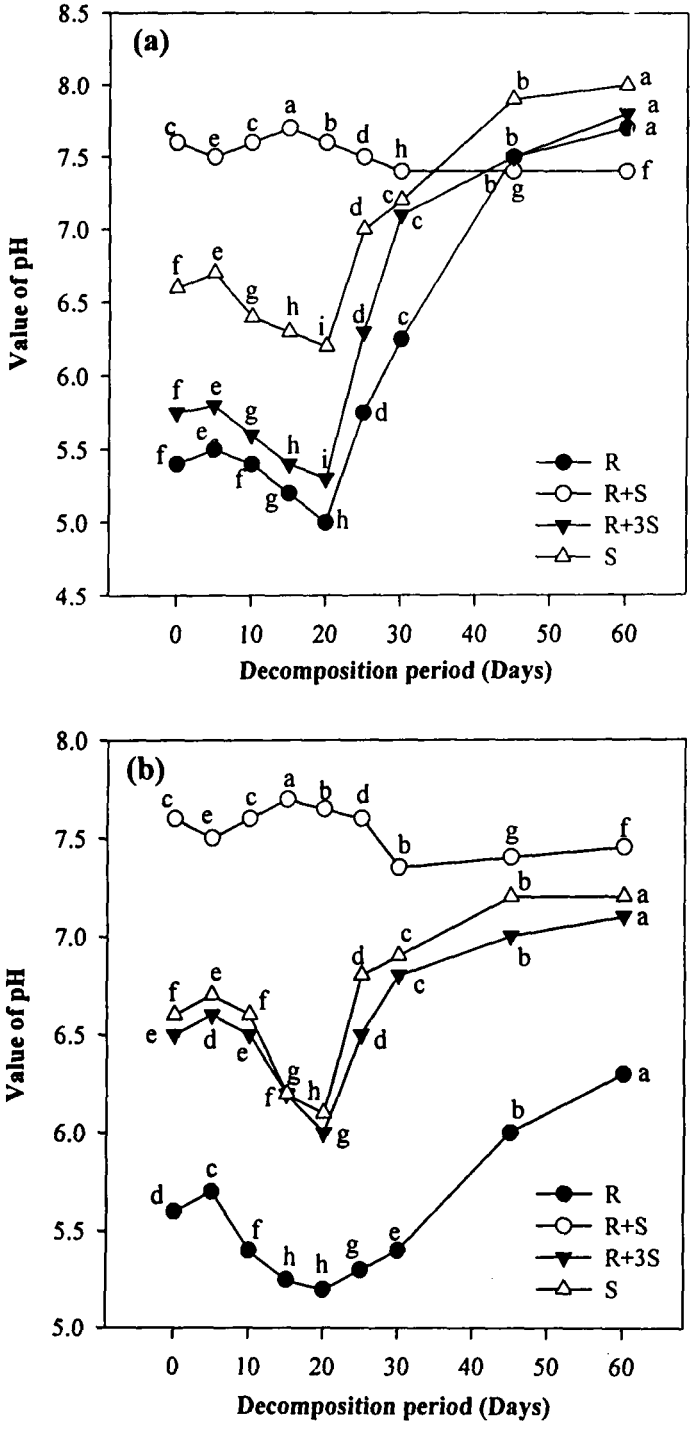
was almost same irrespective of time of decomposition (Fig. 7.3a, b).

c) Changes in pH and Conductivity during Decomposition

In general, pH values decreased with the increase in phytotoxic effect and *vice-versa.* Minimum pH was observed in residues (alone) extracts than others. In both leaves and roots, pH gradually decreased (although insignificantly) and was around 5 (Figs. 7.4 a, b, respectively) during early stages of decomposition. However, during later stages, a significant increase in pH was observed. In. R+S treatment, pH values were more than that of residue (alone) extracts. Here leaf extracts were found to be more acidic than corresponding root extracts. After 20 days of decomposition pH values increased significantly till the end of decomposition. Likewise, pH of R+3S extracts decreased significantly both in leaves or roots during early stages of decomposition. However, at later stages, extracts were either neutral or near neutral in both cases. In extracts of soil, there was no significant change in pH during whole period of decomposition (Fig. 7.4 a, b).

The conductivity was measured to be more in residue extracts and it increased with increasing phytotoxicity. Conductivity of residue extracts was the maximum (irrespective of period of decomposition and part used) followed by conductivity of residues mixed in soils. Minimum conductivity was observed in soil extracts. In case of residue treatment, comparatively lesser conductivity was observed in leaves (nearly 4 mS) than in roots (nearly 13 mS) at any period of decomposition (Fig. 7.5 a, b, respectively). In case of R+S treatment, conductivity was less than 3 mS (except for 15 and 20-days of decomposition, where it was more than 3 mS) when leaves were used as residue (Fig. 7.5a) whereas it was more than 8 mS in roots (Fig. 7.5b).

Fig. 7.4. Changes in pH with time during decomposition of (a) leaves or (b) roots of *A. conyzoides*.



Different alphabets along a curve represent significant difference at $P<0.05$

mS in roots (except in extracts of 45 and 60 day old decomposed material; Fig. 7.5b). The conductivity was nearly 0.2 mS in soil extracts at all stages of decomposition (Fig. 7.5a,b).

Discussion

It is evident from the results that at early stages of decomposition, the phytotoxicity of leaf or root residues increased. It reached maximum on 20 day and started decreasing thereafter. Among all the treatments, the residue (alone) extracts were the most phytotoxic irrespective of the plant parts whereas extracts of soil (alone) were least toxic and there was no effect of decomposition on their phytotoxicity. Moreover, the phytotoxicity of residue extracts was high even after two months of decomposition. However, when these residues were mixed into soil in different proportions, their inhibitory effect gradually decreased especially at the end of the decomposition period.

A similar trend was observed with respect to phenolic content. The amount of total phenolics decreased when residues were mixed in soil. This may be due to amelioration of allelochemicals in soil as their fate is governed by various processes *viz.* utilization by soil microbes (Blum and Shafer, 1988; Blum *et al.*, 1998, 1999; Ravichandran *et al.*, 2005; Thaper and Singh, 2005; Sisodia and Siddiqui, 2007c) their sorption (Huang *et al.*, 1977), and chemical transformation into other toxic or non-toxic compounds (Blum, 1998; Okumura *et al.*, 1999). Phytotoxicity of extracts depended upon amount of residues added. In extracts of R+3S, the phytotoxicity was less than that of R+S. The phytotoxicity as well as phenolic content increased during early stages of decomposition and declined as decomposition time increased (except in residue extracts).

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During decomposition, the residues support a variety of microbial growth, which utilize water-soluble components resulting in higher production of allelochemicals at early stages (Blanco, 2006; Verma and Rao, 2006; Xiaoxing *et al.*, 2006). The prolonged decomposition period results in their microbial breakdown (Harper and Lynch, 1981; Achhireddy and Singh, 1984) and thus phytotoxicity as well as phenolics content declined at the end of decomposition period. However, in case of soil extracts, there was negligible amount of phenolics and decomposition had no effect on phytotoxicity as well as phenolic content. This may be due to the fact that phenolic content in soil is too less to affect plant growth and also production and utilization of phenolics by microbes occur simultaneously. Thus, in present study a correlation between amount of phenolics with time and phytotoxicity was observed during the whole decomposition period which is also supported by a few other workers (Lovett and Ryuntyu, 1992; An *et al.*, 1993; An *et al.*, 2001).

EXPERIMENT – 8

Objective

To study the effect of *Ageratum conyzoides* on nitrogen fixing ability of *Cicer arietinum.*

Hypothesis to be Tested

The growth and development of *C. arietinum* is severely affected by the allelochemicals released from different parts of *A. conyzoides* (as studied in earlier experiments). *C. arietinum* is a nitrogen-fixing crop and consequently, the allelochemicals of *A. conyzoides* may also affect its nitrogen fixing ability. So, the present experiment was undertaken to study the effect of *A. conyzoides* on nitrogen fixing ability of *C. arietinum.*

Parameters Studied

Growth was studied with respect to root, shoot length and plant dry weight of 45-days old *C. arietinum* plants. Nitrogen fixing ability was measured in terms of number of nodules per plant, fresh weight of nodules and amount of leghaemoglobin (hereafter referred as LHb) in the nodules.

Methodology

For the present study, *C. arietinum* were grown in pots filled with rhizosphere soil or soil amended with powders of dried leaves, roots or above-ground parts of *A. conyzoides.* The powders were amended at the rate of 1, 2 and 4 g/100 g soil. For each treatment minimum five replicates were maintained. A similar set of pots filled with unamended control soil (collected from *Ageratum* free area) was also maintained. After 45 days of sowing, root, shoot length and dry weight of *C.*

arietinum plants were measured, Also, nitrogen fixing ability of roots of *C. arietinum* grown in control and amended soil was studied by counting the number of nodules, taking their fresh weight and estimating the amount of LHb (as per Wilson and Reisenauer, 1963) in nodules. The whole experiment was repeated with five replicates each time and the mean data from growth studies was subjected to student's *t*-test, one-way analysis of variance followed by Duncan's multiple range tests.

Results

a) Effect on Growth of *C. arietinum*

A significant reduction was observed on growth of *C. arietinum* plants when grown in rhizosphere soil or soils amended with different parts of *A. conyzoides*. In all pots, more reduction was observed in root length compared to shoot length, irrespective of part amended. Likewise, compared to control, dry weight of *C. arietinum* plants grown in all amended soils was less. Further, the average number of nodules per plant, their fresh weight and LHb content was also observed to be less in rhizosphere soil and powder amended soils as compared to control,

In unamended control soil, root length of 45-days old *C. arietinum* plants was 28.7±1.37 cm whereas in rhizosphere soil of *A. conyzoides*, it was 20.9±1.12 cm. In other words, it exhibited a reduction of 27.68 % compared to control (Table 8.1). In soils amended with any concentration of the powder of any part of *A. conyzoides*, an appreciable reduction in lengths of root and shoot and plant dry weight was seen. The impact increased with increasing concentration. Nearly 10% inhibition was observed in root length at 1 g amendment of leaf or root powder whereas in above ground parts, it was by nearly 18%. However, at highest concentration, maximum reduction of nearly 65% was observed in plants grown in leaf amended soil while in those

grown in root or above-ground part amended soil, nearly 60% inhibition was observed at the same concentration compared to control (Table 8.1). A similar trend of reduction was observed in shoot length; however, the extent of reduction was lesser than in root. Here, about 50% reduction was observed at highest concentration of root powder amendment, while in case of the addition of leaf or above ground parts, nearly 44% reduction was seen in shoot length of *C. arietinum.* When grown in rhizosphere soil of *A. conyzoides*, shoot length of *C. arietinum* plants was 80.5% (13.42±1.22 cm) with respect to that of control (16.68±0.95 cm). However, more reduction was observed in case of those plants grown in soil amended with powder (of any part of *A. conyzoides)* than in rhizosphere soil of *A. conyzoides.* Likewise, the biomass of 45-day old *C. arietinum* plants was also found to be less in amended or rhizosphere soils compared to control soil Like in root and shoot lengths, here also, concentration based reduction was observed. With the increase in the amount of *A. conyzoides* powders in soil, the dry weight of *C. arietinum* plants also decreased. At maximum concentration (4%), nearly 65% reduction over that of control was observed in leaf or root amended soils whereas when powder of the above-ground parts was added to the soil, the biomass of *C. arietinum* was reduced to 58% over that of control (Table 8.1).

b) Effect on Nodulation

Like growth, the nodulation of *C. arietinum* was also affected when it was grown in rhizosphere soil of *A. conyzoides* or soils amended with powders of different parts. There was a deleterious effect on nodulation of *C. arietinum* grown in rhizosphere soil of *A. conyzoides* as there was complete inhibition of nodulation.

Table 8.1 Root and shoot length and dry weight of *C. arietinum* grown in rhizosphere soil or soil amended with leaves, roots and above-ground parts of *A. conyzoides*.

Treatment	Amount of residue in soil (g/100g)	Root length (cm)	Shoot length (cm)	Dry weight (mg)
Unamended soil	0.0	28.7±1.37[a]	16.68±0.95[a]	250.25±8.96[a]
Rhizosphere soil	0.0	20.9±1.12[*]	13.42±1.22[*]	176.43±6.62[*]
Leaves	1.0	25.69±0.65[b]	13.89±0.4[b]	183.52±6.65[b]
	2.0	20.37±1.73[c]	12.83±0.44[c]	145.56±5.48[c]
	4.0	10.05±1.02[d]	9.37±0.67[d]	91.84±2.52[d]
Roots	1.0	26.02±1.15[b]	14.25±1.56[b]	212.71±7.69[b]
	2.0	20.87±1.0[c]	11.83±0.48[c]	138.97±6.52[c]
	4.0	11.07±1.1[d]	8.48±0.72[d]	87.67±3.23[d]
Above-ground parts	1.0	23.65±2.2[b]	13.25±0.68[b]	195.28±8.65[b]
	2.0	19.62±2.32[c]	10.26±0.95[c]	160.99±5.6[c]
	4.0	10.99±1.23[d]	9.21±1.03[d]	103.14±4.36[d]

* represents significant difference from unamended control at $P<0.05$ applying students *t*-test. Different alphabets in a column within each treatment represent significant difference among each other as well as from unamended control at $P<0.05$ applying DMRT.

In control, the average number of nodules counted on roots, were 35.3±6.02 (Table 8.2). The number significantly decreased in *C. arietinum* plants when grown in soil containing increasing amounts of powders of *A. conyzoides*. The number of nodules was almost same in 1.0 g amendment of root or above-ground parts or leaf powder. However, in soil amended with 4.0 g *A. conyzoides* powder prepared from any part there was a complete inhibition of modulation (Table 8.2).

Table 8.2 Average number and weight of nodules and leghaemoglobin content in nodules of *C. arietinum* grown in rhizosphere soil and soil amended with powders of different parts of *A. conyzoides*.

Treatment	Residue amount (g/100g soil)	Average number of nodules (per plant)	Nodule fresh weight (mg/nodule)	Amount of leghaemoglobin (mg/mg nodule)
Unamended soil	0.0	35.3 ± 4.22^{a}	7.76 ± 0.02^{a}	1.47 ± 0.08^{a}
Rhizosphere soil	0.0	0.0	0.0	0.0
Leaves	1.0	30.8 ± 1.27^{b}	6.04 ± 0.02^{b}	0.92 ± 0.04^{b}
	2.0	18.92 ± 1.04^{c}	4.32 ± 0.02^{c}	0.7 ± 0.05^{c}
	4.0	0.0	0.0	0.0
Roots	1.0	31.9 ± 1.41^{b}	6.51 ± 0.01^{b}	0.89 ± 0.07^{b}
	2.0	20.2 ± 1.28^{c}	4.24 ± 0.02^{c}	0.74 ± 0.02^{c}
	4.0	0.0	0.0	0.0
Above-ground parts	1.0	31.4 ± 2.2^{b}	6.8 ± 0.01^{b}	0.92 ± 0.03^{b}
	2.0	23.2 ± 0.81^{c}	4.81 ± 0.01^{c}	0.7 ± 0.05^{c}
	4.0	0.0	0.0	0.0

Different alphabets in a column within each treatment represent significant difference among each other as well as from unamended control at $P<0.05$ applying DMRT.

Further, fresh weight of nodules significantly decreased in amended soils compared to that in unamended control soil. At lowest rate of amendments (1 g/100g soil) of all powders, nearly 17, 16 and 12% reduction in fresh weight of nodules was observed in those grown in soil amended with leaf, root and above ground parts, respectively (Table 8.2). However, in 2% amendment, fresh weight of nodules was reduced by nearly 40% in soil amended with each part of *A. conyzoides.* In contrast, in rhizosphere soil or soil amended with 4 g of *A. conyzoides*, there was complete inhibition of nodule formation (Table 8.2).

Since rhizosphere and powder amended soils severely affected the nodules of

C. arietinum, the amount of LHb in nodules was also determined. It was measured to be significantly less in all amended soils. At the amendment of 1 and 2%, powder of leaf, root or above-ground parts almost equal amount of LHb was estimated in nodules. Maximum (50%) reduction in LHb was observed when *C. arietinum* was grown in soils amended with 2% leaf powder or above ground parts or roots. These observations suggest that *A. conyzoides* besides affecting the growth of *C. arietinum* also inhibits its nitrogen fixing ability by substantially lowering the leghaemoglobin.

Discussion

The present study clearly demonstrates that *A. conyzoides* not only affected the growth and development of *C. arietinum* but also its nitrogen fixing ability. This was reflected through reduced root, shoot lengths and dry weight of *C. arietinum vis-a-vis* number and weight of nodules and leghaemoglobin content in them. There was complete inhibition of nodulation in *C. arietinum* roots when plants were grown in rhizosphere soil of *A. conyzoides*. This may be due to the presence of a number of chemicals that are released by roots or other parts of *A. conyzoides* into the rhizosphere soil, which under natural conditions bring significant ecological effects (Rovira, 1969; Walker *et al.*, 2003; Marinov-Serafimov & Dimitrova, 2007)In rhizosphere soil, however, roots are the major source of interactions, which bring about phytotoxic effect in rhizosphere soil (Bertin *et al.*, 2003b; Bais *et al.*, 2004; Ying *et al.*, 2006; Zhimei *et al.*, 2007). Similar observations were made in soil amended with powders of *A. conyzoides* (particularly at highest rate of amendment) irrespective of its part amended. Plants of *C. arietinum* grown in soil amended with 2% of any part of *A. conyzoides* were devoid of any nodule. The number of nodules and their fresh weights decreased significantly in amended soils. However, it can not

be said that whether the observed effect was due to poor activity of nitrogen fixing bacteria or response of host itself.

Mallik (1999) reported that allelochemicals inhibit biological nitrogen fixing process and thus affect uptake of nitrogen and its metabolism. Almost similar type of observations were also made by (Wardle *et al.*, 1994; Batish *et al.*, 2007) who reported that nitrogen fixation of *Trifolium repens* was severely affected by *Carduus nutans* - an invasive species, by releasing secondary metabolites through its decomposing leaves. Allelochemicals may affect symbiotic nitrogen fixation in legumes by different ways *viz.* affecting legume hosts itself, its microbionts or nodulation processes (Mallik, 1999).

Retardation of growth of *C. arietinum*, its nodulation and nitrogen fixing ability in the present study may be because of accumulation of phenolics-the most common water-soluble allelochemicals (estimated in all parts and rhizosphere soil as indicated in previous experiments) that play a significant role in plant-plant and plant-microbe interactions.

EXPERIMENT – 9

Objective

To identify the putative allelochemicals from different parts of *Ageratum conyzoides.*

Hypothesis to be Tested

In the previous studies, it was observed that *A. conyzoides* exert negative effect on test plants. This was attributed to the water-soluble phenolics released from different parts of *A. conyzoides.* Since phenolics represent a heterogeneous group of compounds of different chemical nature *viz.* coumarins, alkaloids, flavonoids and most common phenolic acids, an attempt was made to identify them. Based on literature survey, the presence of different phenolic acids that play an important role in allelopathy was checked. For the study, above-ground parts, leaves and roots of *A. conyzoides* were used.

Parameters Studied

Phenolic acids were identified through High Performance Liquid Chromatography (HPLC). The retention time (RT) of different peaks from chromatograms obtained from extracts and authentic samples were recorded.

Methodology

Preparation of Extracts for Analysis through HPLC

Ten gram powder of each of the above-mentioned parts was extracted with 80% methanol and shaken on rotary shaker for 24 h at room temperature. The supernatants were separated and their pH was fixed to 2.0 with the help of 2M HCl. The solutions were extracted three times with 50 ml of ethyl acetate. The resultant

solutions were dried and evaporated to dryness on a rotary evaporator at 40 °C.

The phenolic acids were extracted by adding methanol to these condensed residues (obtained after evaporation) in such a way so as to obtain concentration of 1 mg/ml. These methanol extracts of different parts were subjected separately to HPLC for identification. Besides, a dozen of authentic samples of different phenolic acids (Sigma/ Aldrich/ Fluka made) were dissolved in methanol at concentration of 1 mg/ml and run parallel for identification purpose.

Instrumentation

The HPLC was performed on a Shimadzu equipment (LC 10) consisting of LCI OAT binary gradient pumps controlled by class LC 10A software and a Rheodyne type Injection loop. A 25 cm x 4.6 mm reverse phase Shimp-pack C_{18} insert was placed immediately before column. The detection was done using Shimadzu SPD-M10A *VP* tunable UV detector.

Separation Procedure

The optimum separation was obtained with following solvent mixture system

Solvent A: 2% Acetic acid (mobility phase)

Solvent B: 100% Methanol

The separation was programmed in such a way that solvent A constituted 80% while solvent B constituted 20% for whole separation programmed.

Statistical Analysis

The whole experiment was repeated and the mean values of RT with standard deviations presented.

Results

The average values of the RT of samples of extract from different parts of *A. conyzoides* were calculated and compared with those of authentic samples of phenolic acids. In all, eight phenolic acids namely *p*-coumaric acid, gallic acid, ferulic acid, *p*-hydroxybenzoic acid, anisic acid and syringic acid were identified from *A. conyzoides*. However, two phenolic acids remained unidentified. These were a mixture of cinnamic acid and benzoic acid derivatives with varying molecular weights (Table 9.1).

In the above-ground parts, five phenolic acids were detected. These were identified as *p*-coumaric acid (RT = 3.15±0.03), gallic acid (RT = 4.54±0.02), *p*-hydroxybenzoic acid (RT= 9.68±0.08), and anisic acid (RT = 12.30±0.01). Another phenolic acid was also detected at RT value of 14.51±0.04, however, it could not be identified (Table 9.1).

In green leaves of *A. conyzoides,* seven phenolic acids were detected. Of these six could be identified whereas one remained unidentified. The identified phenolic acids included *p*-coumaric acid (RT = 3.15±0.03), gallic acid (RT = 4.54±0.02), ferulic acid (RT = 7.25±0.05), *p*-hydroxybenzoic acid (RT = 9.68±0.08), anisic acid (RT = 12.30±0.1) and syringic acid (18.68±0.08). The RT value of unidentified phenolic acid was 19.95±0.06 (Table 9.1).In contrast to seven phenolic acids identified in green leaves, brown decaying leaves contained only 3. Of these, two were *p*-coumaric acid (RT =3.15±0.03) and *p*-hydroxybenzoic acid (RT = 9.68±0.08). Third phenolic acid at RT 19.95±0.06, which was also detected in green leaves, could not be identified.

Table 9.1 List of different phenolic acids identified from different parts of *A. conyzoides*.

S.No.	Phenolic acid	M.W.	RT	AGP	GL	BL	R
1	*p*-Coumaric acid (4-hydroxybenzoic acid)	164.15	3.15±0.03	+	+	+	+
2	Gallic acid (3,4,5-trihydroxybenzoic acid)	188.14	4.54±0.02	+	+	+	+
3	Ferulic acid (4-hydoxy, 3-methoxycinnamic acid)	194.19	7.25±0.05	-	+	+	+
4	*p*-Hydroxybenzoic acid (4-hydroxybenzene carboxylic acid)	138.12	9.68±0.08	+	+	+	+
5	Anisic acid (4-methoxy benzoic acid)	152.15	12.30±0.1	+	+	+	+
6	Unidentified	-	14.51±0.04	+	-	-	+
7	Syringic acid (4-hydroxy,3,5-dimethoxybenzoic acid)	198.17	18.68±0.08	-	+	-	-
8	Unidentified	-	19.95±0.06	-	+	+	-

In roots, six phenolic acids were identified. These were *p*-coumaric acid (RT=3.15±0.03), gallic acid (RT = 4.54±0.02), ferulic acid (RT = 7.25±0.05), *p*-hydroxybenzoic acid (RT = 9.68±0.08) and anisic acid (RT = 12.30±0.1). The sixth at RT value 14.51± 0.04 (also detected in above-ground parts) remained unidentified.

Discussion

The allelochemicals of *A. conyzoides* were identified to be phenolic acids. In general, *p*-coumaric acid, gallic acid, ferulic acid, *p*-hydroxybenzoic acid, anisic acid and syringic acid were identified from *A. conyzoides*. Among all the parts, maximum

number of phenolic acids was identified from green leaves.The presence of phenolic acids in the different parts of *A. conyzoides indicates* that these play an important role in imparting phytotoxic / allelopathic property to this weed. All the identified phenolic acids are known allelochemicals (Rice, 1984) and have been identified in a number of other allelopathic weeds such as *Parthenium hysterophorus* (Rani, 1990), *Helianthus annuus* (Pariana, 1992), *Lantana camara* (Ambika, 1999), *Croton bonplandianum* (Sisodia and Siddiqui 2008). Their role in allelopathic studies is widely known (Rice, 1984).

Phenolic acids are predominantly found in allelopathic plants and are synthesized within the plants as secondary metabolites. Within plant these remain in glycosidic form to avoid intra-plant toxicity and / or facilitate movement within and outside the plant. These being soluble in water are easily released through leachation from fresh and decaying parts and root exudation besides upon microbial decomposition (Rice, 1984; Ciaxia *et al.,* 2005; Eid and Abou-leila, 2006), Upon release these accumulate in soil in bioactive concentration and undergo a number of changes such as adsorption, chemical transformation, or may even be lost to lower layers through leaching (Blum *et al.,* 1999). Their phytotoxicity in soil thus is a function of their bioactive concentration, which in turn depends upon a number of biotic and abiotic factors (Einhellig, 1996; Kobayashi, 2004). Presence of different phenolic acid in *A. conyzoides* thus indicates that they are responsible for its phytotoxic or allelopathic effect on test plants.

Discussion

DISCUSSION

Ageratum conyzoides is an aromatic annual weed native to tropical America from where it has spread to other tropical and subtropical parts of world. It has now become one of the serious weeds of arable lands in South-east Asia including India and China and severely affects test plants like *Oryza sativa*, *Triticum aestivum* and *Polygonum pleubium*. Though primarily a weed of arable lands, it also infests severely other ecosystems *viz.* grasslands, pastures and forest ecosystems. In agricultural fields this weed is very troublesome and interferes with growth and establishment of test plants affecting the overall productivity. The successful invasion of available niches by *A. conyzoides* can be attributed to a number of characteristics including allelopathy. Recently allelopathy has been proposed to be a novel strategy for the successful invasion in the alien environments by the exotic species at the expense of native communities (Ridenour and Callaway, 2001; Heirro and Callaway, 2003; Bais *et al.*, 2004; Macias *et al.*, 2007). *A. conyzoides* being an exotic weed and greatly interfering with the native communities has not been explored for its allelopathic interference. A study was thus planned to determine the allelopathic interference of *A. conyzoides* with test plants. Series of experiments were conducted for this purpose. The results and observations based on these experiments are discussed here as under:

(a) Rhizosphere Soil of *A. conyzoides* is Phyto-inhibitory in Nature

The present study clearly indicated that growth of various test plants is reduced in rhizosphere soil of *A. conyzoides*. Though the percent emergence of test plants seeds was not affected but there was a significant reduction in growth of

different test plants. Both plant height and biomass of test plants and weeds namely *Anagalis arvensis*, *Brassica oleracea* var. *botrytis*, *Cicer arietinum*, *Triticum aestivum*, *Melitotus alba*, *Phaseolus mungo*, *Oryza sativa* and *Polygonum pleubium* were reduced when grown in *Ageratum* invaded soil. However, magnitude of inhibition varied from plant to plant. In general, maximum inhibition in plant height and dry weight was seen in *Anagalis arvensis* while minimum in *Polygonum pleubium* in case of plant height and *O. sativa* in case of dry weight. These results suggested that soil under *A. conyzoides*, contains some growth inhibitory substances that reduce the growth of test plants. These compounds are released from plant by various mechanisms *viz.* leachation, exudation through roots and decomposition of residues. Among these methods, root exudates are considered as one of the major means of communication between rhizosphere and various microorganisms residing there. The root exudates comprise of a complex mixture of compounds that are responsible for underground interactions (Bais *et al.*, 2004). Besides root-mediated interactions, these may also alter physical and chemical properties of soil and inhibit growth of competing plants (Nardi *et al.*, 2000; Norsworthy and Meehan, 2005; Kong *et al.*, 2006).

Juglone - a phytotoxie compound of *Juglans nigra*, detected in the rhizosphere soil is one of the classical examples indicating that the inhibitors released from the plant accumulate in the soil (Rietveld, 1983). Another example includes the presence of (±) catechin (released from roots of *Centaurea maculosa)* in rhizosphere. It has been observed to be responsible for bringing about inhibitory effects on other surrounding plants (Bais *et al.*, 2002; Corey and Jorge 2008). A strong inhibitory effect of soil collected from surrounding area of *Medicago sativa* plants was studied

by El-Khatib *et al.*, (2003), which indicate the same phenomenon. Kohli (1990) reported the presence of volatile monoterpenes in the rhizosphere soil of the *Eucalyptus* trees. It was speculated that these monoterpenes are contributed by the tree and being heavier than air move downwards and stick to soil particles.

In the *Ageratum* invaded soil, although no single allelochemical was detected as in case of *Juglans nigra* or *Centaurea maculosa*, yet appreciable amount of phenolics was detected that might retard the growth of test plants. The possible role of soil nutrients was ruled out as none of the estimated nutrients was found to be deficient in rhizosphere soil. Further, the pH and other properties of soil also do not point any likely effect on growth of test plants. It is thus, the presence of phenolics in rhizosphere soil that might be reducing the growth of test plants. Whether these phenolics are contributed by root exudates of *A. conyzoides* or from above- or below-ground parts upon leaching remains to be seen.

(b) Different Parts of *A. conyzoides* Exhibit Phytotoxicity

Different parts of *A. conyzoides viz.* above-ground parts, roots, leaves, inflorescence and stem inhibited the germination and growth of *Phaseolus mungo* and thus, exhibited phytotoxicity which differed from one part to other. The extracts prepared from different parts suppressed seedling length and dry weight of *P. mungo.* Seedling length was found to be inhibited more compared to dry weight. Based on our observations, leaves were found to be the most phytotoxic in nature followed by roots and above-ground parts. Compared to this, stem and inflorescence were found to be least phytotoxic. Several other studies have also shown that the different parts of plant exhibit differential phytotoxicity towards other plants (Rice, 1984; Ismail and Kumar, 1996; El-Khatib and Abd-Elaah, 1998; Quayyum *et al.*, 2000; Qasem and Foy, 2001;

Ameena and George, 2002, Turk *et al.*, 2003; Kumar *et al.*, 2006). The maximum inhibitory effect of leaves could be attributed to their proportionally greater biomass and site of synthesis of chemicals. Such observations have also been made by Chon and Kim, 2002; Economou *et al.*, 2002; Xuan *et al.*, 2004; Dana and Domingo, 2006 ; Xiaoqing *et al.*, 2006; Koloren, 2007; Sisodia and Siddiqui 2007a,b.

(c) Phytotoxicity of *A. conyzoides* Changes with Age and Growth Stage

It is clearly indicated from the results that phytotoxicity of *A. conyzoides* differed appreciably during different growth stages and age of plant. For example, extracts prepared from leaves, above-ground parts or roots collected at flowering stage were most phytotoxic in nature compared to other growth stage *viz.* seed stage, vegetative and plantlet stage. During the plantlet stage, the phytotoxic effect of the weed was the minimum and with increasing age, it increased till flowering stage. At seed setting stage, the phytotoxicity declined compared to flowering stage, however, it was more than the vegetative stage. It is thus apparent that the phytotoxicity of *A. conyzoides* is the maximum during the flowering stage. In other words, with increasing age or growth stage of the plant the phytotoxicity changes. The synthesis of phytochemicals and their release from the plant is more during flowering stage, which accumulate in soil in concentrations sufficient to bring a significant effect on the test plants. These observations have also been made by some other workers (Yamane *et al.*, 1992). Studies have shown that production, leachation or allelopathic activity of plants is influenced by plant age, metabolic stage of different parts besides environmental conditions (Weidenhamer, 1996; Josep and Joan, 1997; Pare and Tumlinson, 1997; Agrawal, 1998; Khan *et al.*, 2006; Kong *et al.*, 2008).

(d) Residues of *A. conyzoides* Reduce / Inhibit Growth of Test plants

Ageratum conyzoides grows abundantly especially during its active growing season (between July-March) forming its own monocultures in agricultural fields and other ecosystems. Consequently, it produces large amount of residues under field conditions that also impart a significant phytotoxic nature to the weed. All residues whether leaves, roots or above-ground parts were found to exhibit phytotoxicity towards commonly grown test plants namely *Anagalis arvensis*, *Brassica oleracea* var. *botrytis*, *C. arietinum*, *P. mungo* and *O. sativa.* Leaf residues were particularly more toxic compared to other parts. Both seedling length and seedling dry weight of test plants were reduced by the residues though the response of different test plants was variable, both under laboratory conditions as well as greenhouse conditions, however, the magnitude of phytotoxicity was more since the seeds were directly subjected to extracts in Petri dish bioassay. Under greenhouse conditions, however, the level of phytotoxicity was lesser in soil medium whether residues were amended directly or indirectly in the form of extracts. Though the growth inhibition of various test plants was significant and apparent, yet it was lesser than the laboratory studies. This could be due to amelioration of toxicity in soil or in soil many other factors also contribute like the presence of nutrients in soil, microbial population, and interaction of allelochemicals with the soil particles *viz.* adsorption, chelation or leachation.

A number of allelochemicals get adsorbed with the soil particles either reversibly or irreversibly depending upon the nature of soil and this in turn significantly affects their phytotoxicity (Blum *et al.,* 1999; Kong *et al.,* 2006; Dandelot *et al.,* 2008). Thus, soil is an important medium for the demonstration of allelopathic effect as these conditions are closer to the natural conditions unlike the artificial laboratory

conditions.

(e) Availability of Phenolics is Responsible for Phytotoxic Effect of *A. conyzoides*

In the present study, phenolics - an important group of allelochemicals, were detected in the aqueous extracts prepared either from fresh parts or residues, rhizosphere soil or soil amended with extract or residues of *A. conyzoides.* Their content was the maximum in extracts followed by amended soil and then rhizosphere soil. It was, however, directly related with observed phytotoxic effects on test plants, i.e. greater the amount more was the inhibition. This clearly indicates that phenolics are responsible for the phytotoxic effect of the weed. Phenolics are also known to be present in a number of allelopathic plants, particularly weeds, and play a significant role in community structuring and ecosystem dynamics (Rice, 1984; Inderjit, 1996; Mizutani, 1999; Singh *et al.,* 2001; Castella *et al.,* 2005; Hisashi *et al.,* 2008). Being water-soluble, these are easily released from plants and get accumulated in the soil depending upon the environmental conditions. Phenolics are synthesized within the allelopathic plants as secondary metabolites that are well known for their protective and defensive functions within the plants. Upon, release, however, they play an important role in plant-mediated interactions with, other biotic organisms including allelopathy.

(f) Soil Texture Affects Phytotoxicity of *A. conyzoides*

From our study, it was revealed that soil texture has a significant effect on level of phytotoxicity of *A. conyzoides.* The study involving growth studies with rice *(Oryza sativa)* in soils of different texture amended with leaf powder of *A. conyzoides* indicates that sandy soils, (both sand or sandy loam) inhibited *O. sativa* growth to maximum extent as compared to clay soil, loam or clayey loam. Nearly 87 and 92%

inhibition of *O. sativa* seedling length was observed in sand or sandy loam amended with leaf powder of *A. conyzoides.* This clearly shows that phytotoxicity is influenced by soil texture, which may in turn be affected by the adsorption of allelochemicals to the soil particles. In clayey or clay loams, soil particles being charged, the allelochemicals get adsorbed to these particles either reversibly or irreversibly depending upon various biotic or abiotic conditions. This makes their lesser availability to bring about the growth inhibitory effects on other plants. On the other hand, in sandy soils, allelochemicals hardly adsorb to sand particles and thus remain suspended between different soil particles and are able to bring about significant growth inhibitory effect on the target plants. The greater phytotoxicity in sandy soils was attributed to the availability of more phenolics in these soils in comparison to others. This indicates their non-adsorption or less adsorption to sand particles as pointed earlier. This is an important observation in view of the fact that presence of *A. conyzoides* in sandy soils may bring about significant inhibitions on the other plants, which may be economically important. Precautionary measures can, therefore, be taken to avoid the phytotoxicity of weeds in such soils. The effect of soil texture on phytotoxicity / allelopathy has also been reported by other workers (Einhelling, 1996; Kaushal *et al.,* 2006; Kong *et al.,* 2008).

(g) Decomposition Tremendously Affects Phytotoxicity / Allelopathy

In our study, the phytotoxicity of different parts of *A. conyzoides* put alone or in mixture with soil changed as the decomposition time increased. Initially, phytotoxicity of roots as well as leaves alone or in mixture with soil increased, was the maximum after 20 days of decomposition and declined thereafter. The decrease in phytotoxicity was prominent when residues were allowed to decompose in mixture

with soil. However, when they were put alone for decomposition, the decrease in phytotoxicity was less and it persisted till the termination of decomposition period. This clearly shows that decomposition is enhanced in soil whereas alone it takes more time. A significant correlation was also observed between the level of phytotoxicity of *A. conyzoides* residues and amount of phenolics measured at different stages of decomposition. In other words, amount of phenolics was more in residues exhibiting more phytotoxicity and *vice-versa.* Thus, at the end of the decomposition period, least amount of phenolics was measured and the phytotoxicity of residues was also lesser compared to the other periods of decomposition. The general trend of decomposition was first an increase in phytotoxicity for 20 days followed by a decline. This type of observations has also been reported by other workers studying residue decomposition. In case of *Vulpia* residues, the phytotoxicity of residue extracts decreased as decomposition time proceeded and it was the maximum after 60 days of decomposition and declined thereafter (An *et al.,* 1997), Later, An *et al.,* (2001) studied the kinetics of phytotoxicity of *Vulpia* residues in terms, of identification of allelochemicals and their relationship with phytotoxicity. They reported that the total allelochemical content increased for over a period of 21 days and decreased further. Addition of residues to soil increased production of allelochemicals although these were adsorbed, decomposed, inactivated or even polymerized in the soil. Chou and Patrick (1976) reported that the allelochemical content increased as the *Polygonum pleubium* residues wore subjected to decomposition. It was the maximum after 15-25 days and declined thereafter. In soil alone, no effect of decomposition was observed indicating thereby that soil contains low level of phenolic compounds that is inadequate to bring about any effect on other plants. (Tang and Waiss, 1978; Blanco,

2006) also observed that, there is a relationship between residue decomposition, concentration of allelochemicals and related changes in phytotoxicity as decomposition proceeds.

(h) *Ageratum conyzoides* Affects Nitrogen-fixing Ability of *Cicer arietinum*

In the studies conducted under greenhouse conditions with the amendment of above-ground parts, leaves or roots of *A. conyzoides* in soil, the number of nodules and nodule fresh weight decreased in *C. arietinum* plants compared to those grown in control soil In the rhizosphere soil also, a complete inhibition of nodulation was observed. The complete inhibition of nodulation could be attributed due to the presence of allelochemicals in the rhizosphere soil or soil amended with residues of *A. conyzoides.* Both roots and above-ground parts may release allelochemicals.

However, roots may be the major source of low and high molecular weight compounds released through exudation (Bais *et al.,* 2004). The study thus clearly indicates that allelochemicals released from *A. conyzoides* inhibit or affect the biological nitrogen-fixing process by suppressing the formation of nodules completely or partially. This can be achieved either by affecting legume host itself, the microbiont or the process of nodulation itself (Mallik, 1999). Earlier reports have also indicated that allelochemicals released from different plants affect biological nitrogen fixation of legumes (Rice, 1965, 1968; Rice *et al.,* 1981; Holland and Parker, 1966; Weston and Putnam, 1985; Mallik and Tesfai, 1988; Batish *et al.,* 2007; Alford *et al.,* 2008).

The inhibition of biological nitrogen fixation of *C. arietinum* is a serious matter as nitrogen is one of the important macronutrient and its availability in the form of nitrates and ammonia is often limited and hence has to be supplied either in synthetic form or after fixation either in the form of biological nitrogen fixation or through

abiotic natural factors. However, nitrogen-fixing legumes are the major contributors of nitrogen in the agricultural fields or in the biosphere as a whole. The interference of allelochemicals with this process may lead to harmful effects not only to the test plants but also to the total yield. It is thus important to remove *A. conyzoides* from the agricultural fields to avoid or prevent any adverse effect on test plants particularly on legumes, which additionally fix nitrogen symbiotically.

(i) Allelochemicals of *A. conyzoides* are Phenolic Acids

From the various studies, the nature of allelochemicals was determined to be a group of heterogeneous chemicals, basically comprised of phenolic acids, coumarins, alkaloids, flavonoids and tannins etc. However, phenolic acids are the most common allelochemical group and also known to cause adverse effects on the other plants. In *A. conyzoides*, pher olic acids, namely, *p*-coumaric, gallic, ferulic, *p*-hydroxybenzoic, anisic and syringic, were identified from its different parts and all of these are known to exert allelopathic effect on the other plants. These are very common and have been identified from a number of other weed species. Chou *et al.,* (1998) identified ferulic, vanillic, caffeic and gallic acid, etc, from *Acacia confusa.* Ambika *et al.,* (2003) also identified different phenolic acids from *Chromolaena odorata* - a serious invasive weed in India. Likewise, a number of phenolic acids were also identified from *Fagopyrum esculentum* (Tsuzuki and Dong, 2003), *Polygonella myriophylla* (Weidenhamer and Romeo, 2004), *Vulpia* sp. (An *et al.,* 2001) and *Croton bonplandianum* (Sisodia and Siddiqui, 2008). All these studies indicate that phenolic acids are one of the most common groups of allelochemicals found in a number of allelopathic plants. The reason for their wide spread occurrence could be their release from the donor plant through leachation as they can be easily solubilized in water.

Since these remain in plant in glycosidic form, they do not cause any toxicity to the donor plants. Besides phenolic acids, there are reports that even flavonoids are very common in *A. conyzoides* (Okunade, 2002; runner, a number of volatile allelochemicals namely precocene I, precocene II, 3,3-dimethyl-5-tert-butylindone, β-caryophyllene, fenchyl acetate and γ-bisabolene have also been identified from the volatile essential oil of the weed (Kong *et al.*, 1999). However, in our studies, only phenolic acids were identified since our emphasis was on water-soluble or leachable allelochemicals that accumulate in soil and impart phytotoxicity in it. Based on these observations, made by us and a few others found in literature, it could be concluded that phytotoxicity of *A. conyzoides* could be attributed to a diversity of allelochemicals - phenolic acids.

Conclusions

CONCLUSIONS

From the present study on the phytotoxicity of *Ageratum conyzoides* towards different test plants, following conclusions can be drawn.

- The rhizosphere soil of *A. conyzoides* was found to be inhibitory towards growth of various test plants. Availability of nutrients was not responsible for observed growth inhibitory effect.
- The phytotoxicity of *A. conyzoides* varied with different growth stages of the weed.
- Among different growth stages of weed, the inhibitory effect at flowering stage was observed to be the maximum, whereas that of plantlet stage was the minimum.
- Different parts of *A. conyzoides* exhibited differential phytotoxicity. The fresh green leaves were most phytotoxic in nature.
- Soils amended with either residues or prepared from different parts of weed significantly reduced growth of test plants.
- The aqueous extracts of different parts of *A. conyzoides,* rhizosphere soil of weed and soils amended with its different parts were rich in phenolic allelochemicals.
- Allelochemicals from weed also inhibited nodulation as revealed through the studies on *C. arietinum.*
- The phytotoxicity of *A. conyzoides* was influenced by soil texture and was the maximum in sandy soils.
- During decomposition of different parts of *A. conyzoides* phytotoxicity declined after 20 days.

- In all amended soils, the nutrients were not limiting and hence were not responsible for growth inhibitory effects on plant growth. However, interference of allelochemicals with uptake and transport of nutrients to target plants may be affected leading to indirect effects on plant growth.
- Different phenolic acids *viz.* *p*-coumaric acid, gallic acid, ferulic acid, *p*-hydroxybenzoic acid, anisic acid, and syringic acid were identified from different parts of *A. conyzoides.* In brown (decaying) leaves of *A. conyzoides*, however, only *p*-coumaric acid and *p*-hydroxybenzoic acid were identified.

Future Prospects

FUTURE PROSPECTS

Present study was planned to assess allelopathic effect of different parts of *A. conyzoides* against some test plants. The study revealed some interesting results. However, in order to explore more information on this topic, study can be further elaborated on the following lines:

- To identify allelochemicals in the rhizosphere and amended soils of *A. conyzoides* and to determine changes in nature with time.
- To identify the microorganisms involved in rhizosphere-mediated interactions or during decomposition.
- To study whether weed residues or its pure volatiles and non-volatile allelochemicals possess any selectivity towards weedy species. If so, to evaluate its potential for weed management in different cropping systems.
- To prepare inventory of plants or their genotypes susceptible / resistant to allelochemicals of *A. conyzoides* and to recommend the suitable genotype/cultivars to farmers.
- To prepare mathematical models for dynamics of allelochemicals as a function of concentration and time.

Summary

SUMMARY OF RESULTS

Ageratum conyzoides a native of Central and South America, has now become a dominant species in various tropical and subtropical countries including India. It is a destructive weed of agroecosystems and invades cultivated fields and reduces growth and productivity of plants. Not only can the cultivated fields, the weed even be seen growing luxuriantly in other ecosystems also such as wastelands, grasslands and open unattended areas. All these observations suggest that the weed possesses some interference mechanism, possibly allelopathy that provides selective advantage to it. A study was thus conducted to explore this interference potential. The summary of results is being presented as under:

The plant height and dry biomass of test plants namely *Triticum aestivum*, *Brassica oleracea* var. *botrytis*, *Anagalis arvensis*, *Cicer arietinum*, *Melilotus alba*, *Phaseolus mungo*, *Oryza sativa* and *Polygonum pleubium* grown in rhizosphere soil of *A. conyzoides* were less than those grown in soil collected from the area free of *A. conyzoides* (control soil). However, there was no effect on germination. Further, when these soils were analyzed, rhizosphere soil of *A. conyzoides* was rich in available macro- and micronutrients compared to control soil. The rhizosphere soil contained significantly more amounts of water-soluble inhibitors-phenolics. The inhibitory effect of rhizosphere soil of *A. conyzoides* suggested that the soil contained allelopathic / inhibitory substances in amounts sufficient to suppress the growth of test plants.

In order to find out whether these allelopathic substances are water-soluble or not, specific experiments were conducted. The aqueous extracts prepared from dried

and powdered parts of the weed *viz.* stem, inflorescence, leaves, roots and above-ground parts of *A. conyzoides* were found to be inhibitory towards growth (plant height and dry biomass) of *Phaseolus mungo.* However, the phytotoxicity of extracts differed with different parts. In general, a concentration based effect of extracts was observed in the present study. The aqueous extracts from green leaves were found to be the most phytotoxic followed by roots or above-ground parts. On the other hand, aqueous extracts of inflorescence or stem exhibited lesser phytotoxicity. The aqueous extracts of each part were found to be rich in water-soluble allelochemicals

Further, the allelopathic effect of weed varied with different growth stages *viz.* plantlet stage, bud stage, flowering stage and seed stage. The extracts of different parts collected at flowering stage exhibited the maximum phytotoxic effect towards the growth of *P. mungo* whereas the aqueous extracts of weed collected at plantlet stage caused least phytotoxicity. These results suggested that maximum release of phytotoxic substances occur at flowering stage of the weed. In our study, significantly higher amount of phenolics were found to be present in the aqueous extracts prepared from different parts of *A. conyzoides* collected at flowering stage compared to those collected at any other growth stage of the weed.

Under natural conditions, weed residues / parts get mixed into soil. Therefore, an experiment was performed where the effect of different parts of *A. conyzoides* was assessed in soil. For this, either different concentration of weed residues were directly added into soil or their extracts were mixed into soil. The growth performance of various plants was studied in these amended soils. The early growth of all test plants (*Anagalis arvensis*, *Brassica oleracea* var. *botrytis*, *C. arietinum*, *P. mungo* and *O. sativa)* was severely inhibited when grown in soils amended with different parts of *A.*

conyzoides compared to unamended control soil. More growth retardatory effect was observed in soils amended with higher concentrations of powders or extracts. Further, these amended soils contained higher amounts of available nutrients as compared to control soil. Likewise, the phenolics were also found to be present in higher amounts in amended soils compared to control soil. The growth inhibitory effect of different parts of *A. conyzoides* in soil can thus, be attributed to release of phenolics in the soil. Maximum growth inhibitory effect was observed when residues or extracts of leaves were added into soil.

Since phytotoxicity of *A. conyzoides* may be altered in different textured soils, another experiment was planned where leaf powder was mixed in clayey, sandy, loam, clayey loam and sandy loam soil. In these soils, growth performance of *O. sativa* was observed. The seedling length and seedling dry weight of *O. sativa* was severely reduced in sandy soils amended with even lower concentration of leaf powder of *A. conyzoides.* At highest concentration of amendment, 90% inhibition in growth of seedlings of *O. sativa* was observed in these soils, Compared to sandy soils, phytotoxicity of *O. sativa* was the minimum in loam soil. Likewise, sandy soils mixed with leaf powder were found to contain very high amount of phenolics compared to other amended soils, thereby, indicating their role in retarding growth of *O. sativa.* The amount of phenolics in soils varied with different texture and exhibited a direct relationship with observed growth retardatory effect. For example, more amounts of phenolics were detected where inhibition of *O. sativa* was more and *vice-versa.* Thus, soil texture greatly influences the allelopathic nature of weed.

Under natural conditions, the residues of weed undergo decomposition with time. In order to explore this, weed residues (leaf or root) mixed in soil in different

ratios or alone, were allowed to decompose for two months. The length and dry weight of eight days old seedlings of *Brassica oleracea* var. *botrytis* were reduced to its maximum extent when allowed to grow in extracts of residues alone of either leaves or roots. The phytotoxicity of residues increased significantly for the initial 20 days as reflected through their effect on growth *Brassica oleracea* var. *botrytis*. It declined, thereafter; indicating the completion of decomposition. On the other hand, growth of *Brassica oleracea* var. *botrytis* seedlings remained unaffected in soil alone. Like the phytotoxicity, here also, the amount of phenolics increased initially for 20 days and decreased thereafter. In soil, where no change in growth of *Brassica oleracea* var. *botrytis* was observed, the amount of phenolics was also least. It is, therefore, concluded that during decomposition, significant changes occur in amount and nature of allelochemicals and hence phytotoxic nature of weed changes with time.

Further, the allelochemicals of *A. conyzoides* were also found to inhibit nodulation of *C. arietinum* (an important leguminous crop in India). Number and fresh weight of nodules of *C. arietinum* were significantly reduced, when grown in soil mixed with different parts of *A. conyzoides*. In lower concentrations, where nodulation occurred, leghaemoglobin content was significantly less, compared to control. In rhizosphere soil of *A. conyzoides*, nodulation was completely inhibited compared to control. It is, however, difficult to say whether failure of nodulation is due to lack of root hair formation or inhibition of bacteria responsible for nodulation.

Lastly, attempt was made to identify various allelochemicals found in *A. conyzoides*. Eight phenolic acids were found to be present in organic extracts of different parts of *A. conyzoides* when analyzed through High Performance Liquid Chromatography (HPLC). These included *p*-coumaric acid, gallic acid, ferulic acid,

hydroxybenzoic acid, anisic acid and syringic acid. In green leaves, all these were found to be present while only two *p*-coumaric acid and *p*-hydroxybenzoic acid were present in brown leaves. However, two remained unidentified. Their presence in different parts of weed suggests that these may be responsible for observed growth inhibitory effects on test plants

All these results show that *A. conyzoides* exert inhibitory effect on plants through the release of allelochemicals in soil from its different parts.

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